Monitoring of phenotypic and genotypic changes in antimicrobial resistance in clinical swine bacterial isolates circulating in the U.S.

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Dedication

To my parents for letting me fly and my dogs who through their lives and deaths have taught me more than any human being ever.

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Abstract

Antimicrobial resistance (AMR) has emerged as a global threat to both human and animal health. Use of antimicrobials in food animals has been postulated as one of the drivers for selection of antimicrobial resistance. Recognizing the problem, FDA implemented a feed directive starting January 2017, which stopped the use of antibiotic growth promoters and extralabel use of medically important antimicrobials in food animals. Evaluation of the effectiveness of policy changes such as this feed directive requires long term surveillance of the prevalence of AMR in bacterial isolates of animal origin. However, the current knowledge about AMR in bacteria present in food animals, including swine, is mostly derived from cross-sectional studies and the existing data from NARMS has limited power to evaluate trends at a national level.

Another public health concern is that drugs belonging to certain antimicrobial families of importance for public health, such as those included in the cephalosporin and fluoroquinolone families and are often reserved for secondary line treatments in humans, are used routinely in animal production in certain parts of the world. This use may be selecting for resistance against such critical drugs, thus creating a health risk for the human population. An in-depth knowledge of the molecular mechanisms responsible for AMR can aid in estimating the potential of transmission and the molecular epidemiology of AMR in different bacterial and host populations.

The objectives of this dissertation were to study prevalence and changes in phenotypic and genotypic AMR in swine bacteria of clinical origin collected between 2006-2016 in USA. The results in brief are as follows:

In chapter 2, a detailed systematic review on the global prevalence of resistance to critical antimicrobials in swine *E. coli* was conducted. For colistin, cephalosporins and quinolones, the levels of AMR prevalence were higher in lower-middle income countries as compared to upper income countries. The prevalence of carbapenem resistance was low in most of the countries. Overall, this chapter summarized global prevalence of AMR as well as discrepancies and lack of harmonization in studies worldwide.

In chapter 3, the prevalence and changes in AMR in swine clinical *E. coli* isolates in USA from 2006-2016 was analyzed. The prevalence of resistance to antimicrobials remained constant or changed modestly, except for a drastic increase in enrofloxacin resistance. Multivariate network analysis and rarefaction analysis revealed an increase in the density of AMR networks and an increase in number of combinations of different AMRs (resistotypes), respectively. These results suggest a change in AMR network at individual and multiple AMRs.

In chapter 4, the prevalence and changes in AMR to swine clinical *Streptococcus suis, Pasteurella multocida, Actinobacillus suis and Haemophilus parasuis* isolates in USA from 2006-2016 was analyzed. Where clinical breakpoints or epidemiological cut-offs were available, the prevalence of AMR remained low (<10%) for *S. suis* and *P. multocida.* For *H.parasuis* and *A. suis,* the effect of using surrogate breakpoints from related bacteria from different publications on estimating prevalence and trends was evaluated. The agreement in prevalence estimates for different bacterial-antimicrobial combinations depending on the breakpoint selected varied widely. For bacteria-antimicrobials with no available breakpoints, ordinal regression models indicated a decrease in resistance to 3 antimicrobials for *A. suis* and an increase in resistance to 7

antimicrobials for *H. parasuis.* These findings indicate changes in resistance to certain antimicrobials in swine bacterial pathogens of animal health importance.

In chapter 5, the genetic determinants of extended spectrum cephalosporin and fluoroquinolone resistance were studied in selected swine clinical *E. coli* isolates. Plasmids carrying ESBL-encoding genes and plasmid mediated quinolone resistance genes were assembled and described. Additionally, genes such as *mcr-9* and *fosA7* were identified in swine *E. coli* isolates in USA for the first time. These genes were present on diverse *E. coli* ST types spread throughout Midwest USA, indicative of a change in genetic mechanisms of cephalosporin resistance in swine population in USA.

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CHAPTER -1 Introduction

In the last decades antibiotic resistance has emerged as an extremely important problem affecting veterinary and human medicine. Antibiotics have been the cornerstone of modern medicine against infectious diseases, but it has been speculated that the emergence of antibiotic resistance may lead us to a post-antibiotic era in which common infections and minor injuries would be deadly once again.[1] Use of antibiotics in animal production systems has been implicated as one of the causes for the increased prevalence of resistant bacteria in both animals and humans, and thus, it is considered a major public health issue.[2] In food production systems antibiotics can be not only used for therapeutic purposes but also for prophylaxis and as growth promoters.[3] It has been estimated that roughly 70% of all antibiotics produced in the world are consumed in food animal production systems.[4] Moreover, several studies worldwide have reported the existence of bacteria isolated from animal production systems that are resistant against critical drugs used in human medicine, such as cephalosporins and fluoroquinolones.[5]

Taking note of the impact of antibiotic use in animal production systems on AMR emergence, the FDA has recently taken initiatives to phase out the use of medically important antibiotics as growth promoters in animal production in the US, effective from January 2017.[6] Restriction on the use of growth promoters is expected to contribute to a decrease in the prevalence of antibiotic resistant bacteria, extensively drug resistant and pandrug resistant bacteria in food animals.[7] However, to evaluate the success or failure of such policies information about the baseline antimicrobial resistance levels is critically needed. Unfortunately, except for surveillance conducted under the National

Antimicrobial Resistance Monitoring System (NARMS), performed on a relatively small number of isolates of certain bacterial species, there is little information available regarding the current status of antimicrobial resistance in bacteria from food animals. In addition to the dedicated active surveillance systems, passive data available from diagnostic labs, such as Minnesota Veterinary Diagnostic Lab (MVDL), can be used to analyze patterns of antimicrobial resistance in food production systems.[8] In fact, Mather et al (2016) have suggested that passive surveillance using diagnostic based data might be more effective in detecting rare and emerging resistant phenotypes.[9]

Previous field and in-vitro studies have suggested that resistance against one antibiotic may select for other unrelated antibiotics.[10] For example, a recently published study demonstrated a statistical association between use of tylosin and tulathromycin in co-selection and persistence of chloramphenicol at Irish pig farms.[11] Moreover, with an increase in available computational strength and new statistical models, co-resistance can be analyzed in novel ways.[12] Currently, NARMS analyzes data at individual AMR level only.

At a genetic level, resistance to a particular antimicrobial might be conferred by different mechanisms e.g. chromosomal mutations, horizontal transfer via plasmid-medianted resistance genes, etc.[13] Understanding the genetic mechanisms behind AMR is crucial as different mechanisms might exert different fitness costs on bacterial cells. Recently, it has been characterized that colistin resistance in swine bacterial isolates can be induced by a stable, transferable plasmid carrying the *mcr-1* resistance gene, whereas previously colistin resistance was only associated with an unstable chromosomal mutation.[14] Moreover, different mechanisms of fluoroquinolone resistance have been shown to exert

different fitness costs on host bacteria. For example, in one experimental *in-vitro* study, the presence of a PMQR gene *qnrA1* was found to decrease fitness by 9-21 %, whereas the presence of *qnrS1* led to an increase in bacterial fitness by 7-21 %.[15] Hence, knowledge of the molecular mechanisms behind phenotypic resistance might aid in understanding the emergence of specific AMRs and, when coupled with *in-vitro* and/or *in-vivo* studies, predict the success or failure of available mitigation measures.

The US swine industry has grown rapidly over the last two decades in terms of pig crop as well as pigs per breeding animal, among other parameters.[16] Minnesota accounts for nearly 12 % of the national hog and pig inventory, ranking third after Iowa and North Carolina.[16] However, due to changes in consumer perception about antimicrobial use in food animal production systems,[17] antimicrobial use in animal production including swine is under scrutiny. Some authors even suggest different taxation systems for antimicrobial-fed and antimicrobial-free meats.[18] Hence, there is a critical need to assess the levels of antimicrobial resistance and its association with antimicrobial use in the swine industry from both animal health, human health and economics perspectives.

The fact that dissemination of AMR is not restricted to a particular country further complicates this critical threat. International travel,[19] food trade[20] and role of migratory wildlife[21] have all been implicated in transmission of bacteria resistance to critical antimicrobials across international borders. Recognition of AMR as a global threat rather than a regional one has been one of the key reasons behind setting up the Global Antimicrobial Resistance Surveillance system (GLASS) by WHO.[22] The aim of this surveillance program is to standardize the reporting of AMR to key antimicrobial-

bacteria combinations globally, and collect and update the current status of AMR. Currently, the focus of this program is surveillance of human pathogens. The current knowledge about global prevalence and trends in AMR to critical antimicrobials in bacteria fromfood animal species remains unknown. A report on the current global status of AMR in a particular food animal species (such as swine) will complement the fledgling efforts of GLASS.

Objectives

The objectives of this dissertation were to:

1. To systematically collect and appraise information on the global prevalence of phenotypic and genotypic resistance to certain critical antimicrobials in isolates of indicator bacteria (*Escherichia coli*) collected globally from various sources of swine origin
2. To analyze the prevalence and changes in resistance to a panel of antimicrobials in a pathobiont of both human and animal health importance (*Escherichia coli*) collected from diseased pigs in USA
3. To determine the prevalence and changes in resistance to a panel of antimicrobials in pathogens involved in respiratory disease complex and polysystemic infections in swine in USA (*Streptococcus suis*, *Pasteurella multocida*, *Actinobacillus suis* and *Haemophilus parasuis*)
4. To ascertain the genomic basis of extended spectrum cephalosporin and fluoroquinolone resistance in swine *E. coli* isolates collected from diseased pigs in USA

CHAPTER-2 Global Prevalence of Extended Spectrum Cephalosporin, Carbapenem, Colistin and Quinolone Resistance in Escherichia coli of Swine Origin - a systematic review and meta-analysis

Introduction

The threat posed by the emergence of antimicrobial resistance (AMR) worldwide has led to an increased focus on monitoring both antimicrobial use (AMU) and AMR in public health. Undoubtedly, a high percentage of antimicrobials produced worldwide are consumed by animals,[23] representing a possible driver and source of AMR that could impact both animal and public health. Although the link between AMU and development of AMR in animal production has been well demonstrated,[24,25] the full impact of AMU on public and animal health is still not fully understood. To advance understanding of the complex epidemiology of AMR, it is necessary to monitor AMR in both domestic animals and humans, and many national and regional AMR monitoring systems include an animal and/or animal-based food component.[26–28] Antimicrobial usage is particularly common in more intensified animal production systems, such as poultry and swine.[29] In these animal species antimicrobials have been used to promote growth, for prophylaxis, metaphylaxis and as therapeutic agents against a multitude of bacterial pathogens,[3] some of which are foodborne zoonoses.

The levels of concern associated with antimicrobial-resistant bacteria is influenced by the medical importance of the respective antimicrobials. The World Health Organization (WHO) has classified certain antimicrobial classes as being critically important for human medicine. These include third and higher generation cephalosporins, colistin, carbapenems and fluoroquinolones among others classes of antimicrobials.[30]

Some antimicrobials belonging to these classes, are also used in veterinary medicine, even though using these antimicrobials in animals could potentially lead to cross-selection of genetic determinants of resistance to related antimicrobials used in human medicine.[31]

Resistance to extended-spectrum cephalosporins is mediated by extended spectrum beta-lactamases (ESBLs), (commonly encoded by the *bla*$_{\text{TEM-, CMY-, SHV-, CTX-M-}}$ family of genes) and plasmidic AmpC (commonly encoded by the *bla*$_{\text{CMY}}$ family of genes).[32] The epidemiology of AMR is complicated by the numerous genes that encode for resistance to antimicrobials in the same or different antimicrobial class. For example, ESBL genes (like *bla*$_{\text{VIM-, IMP-, OXA-48, NDM-}}$) encode resistance determinants to various cephalosporins and carbapenems,[33] and might show variable substrate specificity.[34] Moreover, some of these resistance mechanisms are either novel or have been discovered only recently. Recent discoveries of plasmid mediated genes encoding colistin resistance (*mcr*) are another example of the constantly evolving threat of AMR, reinforcing the need for genomic epidemiology in AMR surveillance.[14] Similarly, resistance to nalidixic acid (NAL) and fluoroquinolones (FQs) was thought to be mediated by chromosomal mutations and increased activity of efflux pumps.[35] However, in the last two decades plasmid-mediated mechanisms of quinolone resistance (PMQRs) have been increasingly reported, making the genomic basis of FQ- and NAL-resistance even more complex than initially considered.[36]

Escherichia coli is a commensal bacterium and potential pathogen found in the intestinal microflora of animals and humans. *E. coli* is favored as an indicator bacterium for AMR surveillance because of its ubiquitous nature, ease of isolation and identification

and propensity to acquire and disseminate resistance carrying plasmids.[37] This bacterium is also easily isolated from water, environmental samples and raw animal-based products and hence has been used extensively as an indicator of AMR across the food chain. For example, presence of similar genetic determinants of AMR in *E. coli* isolated from humans and food animals have been considered evidence of transfer of AMR across the food chain.[38]

Because of the widespread interest in AMR among clinicians, veterinarians and public health practitioners, there has been an exponential increase in the number of scientific publications focusing on this topic, particularly in recent years. The objective of this systematic review was to describe geographical distribution and temporal changes in the prevalences of phenotypic and genotypic resistance to critically important antimicrobial classes (third and fourth generation cephalosporins, carbapenems, colistin, quinolones) in this widely used indicator bacterial species among isolates from swine, a food animal species in which antibiotics are used extensively worldwide.

Methods

Literature search

Four databases (PubAg, Web of Science, Pubmed and CAB abstracts) were selected after consultation. The search strings employed have been provided in supplementary material (S2-1). All the databases were initially searched on April 13, 2017 and updated on July 27, 2018. References were retrieved and imported to Mendeley (Mendeley Ltd., version 1.17.13) and duplicate articles were removed. Additionally, reports on AMR in *E. coli* from swine and/or pork not indexed in scholarly databases were identified from the webpages of national and international agencies coordinating surveillance programs for

antimicrobial resistance (EFSA-European countries, DANMAP-Denmark, MARAN- The Netherlands, NORMVET-Norway, SVEDRES-Sweden, FINRES-Finland and CIPARS-Canada).

The relevance of the retrieved references (titles and abstracts when available) was first screened by three reviewers by determining if there was a mention to antimicrobial resistance, *Escherichia coli* or *Enterobacteriaceae*, and livestock in general. The full-texts of citations retained at this stage were then retrieved and subjected to a second screening in which relevant data were extracted (see below). Data were collected from articles meeting the following three inclusion criteria:

1. There was data on prevalence of genotypic and/or phenotypic antimicrobial resistance cephalosporins, FQ, NAL, colistin and/or polymyxin E

2. Resistance was measured in *Escherichia coli*

3. *E. coli* were retrieved from pigs and pork or meat products of porcine origin (hereon referred to as "PMP")

Studies were excluded if the articles were written in a language other than English; if they provided no original data (review articles); if they were experimental in nature (drug trials under experimental conditions, manipulation of plasmids etc.); if information was not provided for *E. coli* or bacteria were not isolated from pigs/PMP; if a denominator (number of isolates tested) from which the resistant isolates were obtained was not provided; if bacterial isolates were not clearly classified as resistant/non-resistant by the authors or information to classify them as such was not provided; or if the articles were focused on antimicrobial usage without providing information on AMR. Articles

were reviewed and data were extracted at this stage by lead reviewer, except for data from articles published from 2000-2006 which were extracted by another reviewer.

Data extraction

Data from articles retained after the application of inclusion/exclusion criteria were compiled into a Microsoft Excel spreadsheet. Data extracted included year of study, first author's last name, country in which the study was conducted, health status of the studied pig population, type of samples from which the bacteria were recovered, methodology used to assess bacterial antimicrobial susceptibility, antimicrobials tested, criteria for classifying bacteria as resistant/non-resistant, total number of isolates tested and number of isolates resistant to each antimicrobial.

Statistical analysis

In our literature search, prevalence of genetic mechanisms encoding for cephalosporin resistance was studied either in *E. coli* populations collected without any bias of antibiotic enrichment or phenotypic resistance (hereon referred to as "general-EC") or in ESBL-*E. coli* (ESBL-EC) populations. ESBL-EC were defined in the studies based on phenotypic methods suggested by CLSI or presence of genes conferring ESBL phenotype. Countries were classified as upper income countries (UIC) if these belong to high income class, or lower-middle income countries (LMIC) if these countries were in upper-middle income, lower-middle income or low-income classes, based on World Bank classification.[39] Pooled prevalences of specific AMR (cefotaxime, ceftiofur, ceftazidime, colistin, fluoroquinolones and nalidixic acid) in general-EC and ESBL-EC prevalence were calculated at the country level, and separately for isolates collected from healthy pigs, diseased pigs and PMP. Pooled prevalences were also estimated for *bla* and *mcr-1*

genes in general-EC at a country level. Proportions of resistance to each antimicrobial were first transformed using Freeman-Tukey double arcsine transformation, and pooled estimates were then calculated using a random effects model using empirical Bayes estimator and back-transforming the estimates.[40] Crude percentage prevalences were reported for *bla* and *qnr* genes in ESBL-EC and phenotypic carbapenem resistance in general-EC because of lack of sufficient number of articles and/or isolates. These country-level estimates were classified as very low (<1%), low (1.1-10%), moderate (10-20%), high (20-50%), very high (50-70%) and extremely high (70-100%) as per EFSA guidelines.[41]

To study changes in the prevalence of resistant general-EC and ESBL-EC over time, hierarchical multivariable generalized linear mixed models were fitted using restricted maximum-likelihood estimation (REML). In these models, raw percentage of resistance to individual antimicrobials (cefotaxime, colistin, ceftiofur, ceftazidime, FQ and NAL) in general-EC or ESBL-EC prevalence at the study level were considered as the outcome variables; time (in years), income levels of countries and source (healthy pigs and PMP vs diseased pigs) as fixed effects and country as hierarchical random effect. Data from pigs of unknown clinical status were pooled with the data from healthy pigs for meta-analysis. For the articles where data were collected over several years, the midpoint of the study period was used in the analyses. For example, if data were collected between 2008 and 2012 and information about individual years was not provided, then 2010 was considered as the time point in the analysis. Some articles did not provide data on the year of sample collection. For these articles, an estimated time-point of sample collection was calculated by a linear regression model based on articles

with complete information on year of sample collection and year of publication. Meta-analyses were performed using 'metafor' package in RStudio version 1.0.136 using random-effects regression model.[42,43] Choropleth maps were made using QGis Version 2.18.16.

Results

Literature search

The initial search conducted on the four databases retrieved 4,037 references. Additionally, data from 70 surveillance reports from national and international agencies were also included in the literature review. After removal of duplicate articles, the relevance of 2,869 references was assessed through the screening of their title and abstracts. Of these, 1,082 references were deemed relevant and their full texts retrieved and subjected to the second screening (Figure 2-1). Finally, data from a total of 324 articles included in this literature review were extracted.

Characteristics of articles included

The characteristics of articles included in the final stage of the review (n=324) are presented in table 2-1. The studies included were published between 1983 and 2018, with nearly 62% of them being published after 2010. Articles contained data about isolates collected from as early as 1974 and as recently as 2018. Overall, data on resistance on *E. coli* recovered from pig or PMP from 55 countries was retrieved. Most of the articles selected reported data from European (158 articles, 48.8%) or Asian (101 articles, 31.2%) countries. All except two articles (n=322) contained data on isolates recovered from pig samples, and 62 articles included data on isolates on PMP. Among the studies of *E. coli* from pigs, some health characteristics were provided in 283/324 (87.3%). Phenotypic

resistance data were available from 300 articles and were predominantly derived from dilution methods (agar dilution or broth microdilution) (n=173, 57.7%) and disk diffusion (n=124, 41.3%) methods. In the 266 articles containing data about cephalosporin resistance and published after 2000, 163 and 53 articles used the CLSI breakpoints or guidelines issued by EUCAST, respectively. Forty articles did not specify the interpretive criteria used to categorize isolates as susceptible/resistant. Since the interpretive criteria for colistin and carbapenem were introduced only in the last decade, many authors used manufacturer-based cut-offs or cut-offs suggested by other scientists. Hence, use of interpretive criteria was not evaluated for carbapenem and colistin resistance.

Prevalence of phenotypic and genotypic antimicrobial resistance

Very low to low (<10 %) prevalences of resistance to third generation cephalosporins (ceftazidime, ceftiofur and cefotaxime) were estimated for general-EC isolates collected from healthy pigs, diseased pigs and PMP in Europe, with the exception of ceftiofur resistance in isolates collected from diseased pigs in Spain (10.6%) and Romania (18.0 %) (figures 2-2, 2-3, 2-4, 2-5, 2-6; tables 2-2, 2-3, 2-4).

By comparison, the prevalences of resistance to ceftiofur were more heterogenous in general-EC isolates collected from Asia (figures 2-2, 2-3; table 2-2). While these prevalences were very low to low (<10 %) in isolates collected from healthy and diseased pigs from Japan, South Korea, Taiwan, Thailand and Vietnam, moderate to high prevalences of ceftiofur resistance were estimated in general-EC isolates collected from China (21.0%, healthy pigs; 30.3%, diseased pigs).

In North America and Australia, prevalences of ceftiofur resistance were very low to low (<10%) in general-EC isolates from all 3 sources collected from Australia, Canada and the United States of America (USA), except for moderate prevalences of ceftiofur resistance in general-EC isolates collected from PMP in USA (12.0%) (figures 2-2, 2-3; table 2-2). Low prevalences of ceftiofur resistance were estimated from general-EC isolates collected from healthy pigs in South America (Argentina-6.1%; Brazil-2.6%). However, 14.9% of the general-EC isolates collected from diseased pigs in Argentina were resistant to ceftiofur (figure 2-3; table 2-2).

Moderate to high prevalences (10-50%) of cefotaxime resistance were estimated for general-EC isolates collected from China (12.3%, healthy pigs; 18.8%, diseased pigs), India (28.7%, healthy pigs; 36.1%, diseased pigs), Thailand (32.7%, healthy pigs; 10.5% diseased pigs) and South Africa (14.5%, healthy pigs) (figures 2-4, 2-5; table 2-3). In Nigeria, 73.7% of general-EC isolates collected from healthy pigs were ceftiofur resistant (figures 2-4, 2-5; table 2-3). Other non-European countries that had lower prevalences (<10%) of cefotaxime resistance in general-EC isolates collected from healthy and diseased pigs were Australia, Cambodia, Canada, Chile, Cuba, Grenada, Japan, South Korea and Taiwan (figures 2-4, 2-5; table 2-3).

Among the general-EC isolates collected from healthy and diseased pig sources, prevalences of ceftazidime resistance were low (<10%) in some Asian countries (Cambodia, South Korea, Taiwan and Vietnam), Argentina, Australia and Grenada (figure 2-6; table 2-4). Resistance to ceftazidime was extremely variable in general-EC isolates

collected from China (2.26%, healthy pigs; 12.4%, diseased pigs), Thailand (18.5%, healthy pigs; 3.61%, diseased pigs) and Nigeria (74.5%, healthy pigs) (figure 2-6; table 2-4). An extremely high prevalence of ceftazidime resistance was observed in general-EC isolates collected from meat products in Egypt (62.7%) and diseased pigs in India (99.9%) (figure 2-6; table 2-4).

Pooled prevalence of ESBL-EC was consistently very low to low (<10 %) in European countries, except for isolates from healthy pigs in Portugal (14.3%) (table 2-5). Again, a greater variability was observed in studies from Asian countries, in which prevalences of ESBL-EC were found to be very low to low (Japan and South Korea), moderate to high (Cambodia, China, India, Vietnam and Taiwan) and very high to extremely high (Hong Kong and Thailand) depending on the country and the source (table 2-5). Higher prevalences of ESBL-EC (25.1-43.2%) were estimated in isolates from diseased pigs from China and India compared to isolates from healthy pigs (ranging between 18-20 %) (table 2-5), whereas the opposite was observed in Thailand, where ESBL-EC prevalences were higher for isolates from healthy pigs (48.9%) as compared to diseased pigs (12.0%) (table 2-5). The prevalences of ESBL-EC in isolates from PMP were also very variable, ranging between low prevalence in Japan (2.79%) to higher prevalence in Vietnam (31.9%) (table 2-5). From the Americas and Africa, only estimates from Brazil and Nigeria were available, where the prevalence of ESBL-EC resistance was low (2.61%) and high (31.5%), respectively (table 2-5).

Pooled prevalences of *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{CMY}$ and *bla*$_{TEM}$ genes in general-EC populations were estimated (table 2-6). Overall prevalences of *bla*$_{SHV}$ and *bla*$_{CTX-M}$ genes were below 10% in all countries with data available (table 2-6). Pooled prevalences of *bla*$_{CMY}$ were similarly low in these populations with the exception of USA, where nearly 38.4% of general-EC isolates carried *bla*$_{CMY}$ genes (table 2-6). Pooled prevalences of *bla*$_{TEM}$ were low (<10%) in isolates from Bulgaria, Canada and India (table 2-6). In contrast, pooled prevalences of *bla*$_{TEM}$ were moderate to high (14.4-49.6%) in general-EC isolates collected in China, Czech Republic, Denmark, Poland and USA (table 2-6). Notably, general-EC isolates from Australia (55%) and Thailand (94.1%) had very high to extremely high prevalence of *bla*$_{TEM}$ genes (table 2-6).

Raw percentage prevalences of *bla* genes in ESBL-EC populations are presented in table 2-7. Briefly, the prevalences of *bla*$_{SHV}$ genes were nearly zero percent in 6 countries from which data was available for more than 20 isolates (Canada, China, Germany, Taiwan, Thailand and Vietnam) (table 2-7). However, nearly 9%, 16%, 33% and 62% of United Kingdom (UK), Belgian, Spanish and South Korean ESBL-EC isolates carried these genes, respectively. The distribution of *bla*$_{CMY}$ genes among ESBL-EC was highly variable, ranging from 1.89% to 96.5% across ESBL-EC isolates collected from different countries (table 2-7). Similarly, the distribution of *bla*$_{TEM}$ genes among ESBL-EC isolates was also extremely variable, ranging from 27.0% to nearly 100% among ESBL-EC isolates from Germany and South Korea, respectively (table 2-7). With the exception of 3rd generation cephalosporin resistant isolates from Canada, more than 20% of the ESBL-EC isolates from other countries with sample sizes of greater than 20

isolates carried *bla*$_{\text{CTX-M}}$ gene (table 2-7). More than 80% of ESBL-EC isolates from Belgium, Germany, Japan, The Netherlands, Taiwan, UK and Vietnam carried this group of genes (table 2-7).

At least 21 different *bla*$_{\text{CTX-M}}$ alleles were reported across general and ESBL-EC populations across all studies (table 2-8). ESBL-EC isolates from China carry the most diverse repertoire of *bla*$_{\text{CTX-M}}$ alleles, with at least 14 different *bla*$_{\text{CTX-M}}$ alleles reported so far (table 2-8). Among the European countries, ESBL-EC isolates from UK carried 7 different allele types, followed closely by Germany with 5 different allele types (table 2-8). *bla*$_{\text{CTX-M-1}}$ was the most widespread allele, with nearly 59% of ESBL-EC from 12 countries carrying it (table 2-8). Other notable genes prevalent worldwide were *bla*$_{\text{CTX-M-9}}$ (32.5%), *bla*$_{\text{CTX-M-14}}$ (16.6%), *bla*$_{\text{CTX-M-15}}$ (12.4%), *bla*$_{\text{CTX-M-55}}$ (18.5% in Asian isolates) and *bla*$_{\text{CTX-M-65}}$ (12.1%) (Table 2-8).

Overall prevalences of carbapenem resistance were found to be extremely low (0.48%, n=65/13,451 general-EC isolates collected from 39 countries, table 2-9). Carbapenem resistant general-EC isolates were reported from Australia (0.39%, n=2/519), China (1.37%, n=11/805), Germany (0.89%, n=4/450), Greece (3.59%, n=6/167), India (20.5%, n=23/112), South Africa (1.78%, n=3/169), South Korea (0.77 %, n=1/130), The Netherlands (0.14%, n=3/2159), Portugal (0.37%, n=1/264) and Thailand (0.38%, n=2/520) (table 2-9). Additionally, in a study focusing on general-EC isolates from European countries, 0.14% (11/7850) of isolates were carbapenem resistant.[44] While none of the ESBL-EC isolates from healthy pigs in Germany (n=0/211), Japan (n=0/22), Thailand (n=0/134) and Vietnam (n=0/39), 28.6 and 1.9

percent of the ESBL-EC isolates collected from animals in Brazil (n=8/28) and South Korea (n=9/469) were phenotypically carbapenem resistant, respectively.[45–52] None of the 79 colistin resistant isolates from sick pigs tested in France were carbapenem resistant.[53]

Additionally, genes coding for carbapenem resistance were also found in general-EC isolated from healthy pigs in Germany (*bla*$_{\text{VIM-1}}$, 2/238, *bla*$_{\text{OXA-48}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{KPC}}$=0/238) and diseased pigs in China (*bla*$_{\text{IMP-1}}$, 1/315).[54–56] Genes encoding carbapenem resistance encoding genes were not found in a limited number of general-EC isolates isolated from different sources USA (n=199, genes tested= *bla*$_{\text{NDM-1}}$) or Denmark (n=54, genes tested= *bla*$_{\text{VIM-2}}$, *bla*$_{\text{IMP-2}}$).[57,58]

In South Korea, a large spectrum of genes encoding for carbapenem resistance were found in ESBL-EC isolates collected from healthy pigs (*bla*$_{\text{NDM-1}}$-14.7%, *bla*$_{\text{KPC}}$-12.6%, *bla*$_{\text{VIM}}$-5.3%, n= 95).[49] In Germany, a single isolate out of 221 ESBL-EC isolates collected from healthy, fattening pigs was found to be harboring *bla*$_{\text{VIM-1}}$ gene.[59] Additionally, among carbapenem resistant isolates collected in China, all 5 and 9 isolates possessed *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{NDM-5}}$, respectively in different studies.[60,61] Eight out of 23 Indian and 2 out of 11 European carbapenem resistant isolates carried *bla*$_{\text{NDM-1}}$ and *bla*$_{\text{OXA-181}}$ genes, respectively.[44]

In Europe, prevalences of colistin resistance ranged between very low and low in general-EC isolates from all sources, except Spanish and Serbian isolates from diseased pigs, in which higher prevalences of resistance (23.4-33.8%) were found (figures 2-7, 2-8; table 2-10).

In Asian countries, very low (<1%) and moderate-high (16.6-34.5%) prevalences of colistin resistance were reported among general-EC isolates from healthy and diseased pigs in Japan and South Korea, respectively (figure 2-7, 208; table 2-10). Moderate to high prevalences of prevalence (11.7-31.2%) of colistin resistance were found in general-EC isolates from both healthy and diseased pigs in Cambodia, China, Thailand, and Vietnam (figure 2-7, 2-8; table 2-10). Prevalences of colistin resistance were very low and high in general-EC isolates collected from healthy pigs in Zambia (0.26%) and diseased pigs in Brazil (23%), respectively (figure 2-7, 2-8; table 2-10).

Additionally, none of the ESBL-EC isolates from healthy pigs and tested for phenotypic resistance in Thailand (n=80) and UK (n=190) were colistin resistant.[52,62] However, 6.5% of the 214 ESBL-EC isolates from healthy pigs in Italy were colistin resistant.[63] In ESBL-EC isolates from China, none of the 198 isolates from diseased pigs and 36.1% (n=35/97) of the isolates from healthy pigs were phenotypically tested to be colistin resistant.[64,65] While our search revealed no general-EC or ESBL-EC isolates that were tested for colistin resistant in Taiwan, 45.9% (n=161/351) of the florfenicol resistant isolates were colistin resistant.[66]

Globally, a total of 475 (2.66%) of the 17,838 general-EC isolates collected between 2015-2018 from different sources harbored *mcr-1* (table 2-6). The pooled prevalences of *mcr-1* gene in general-EC population were very low (<1%) in 10 out of 15 countries spread across three continents, low in 3 countries (Japan, Spain and Venezuela) and moderate in 2 countries (China and Vietnam) (table 2-6).

Presence of *mcr-2* was studied in 2,519 general-EC isolates collected from healthy and diseased pigs in China, Italy, Japan and Spain but none of the isolates carried this gene.[62,63,67,68] Similarly, none of the 299 general-EC isolates from diseased pigs from China and Spain carried *mcr-3*.[63,67,69] None of the 169 general-EC isolates collected from healthy pigs in Italy carried *mcr-3, mcr-4* and *mcr-5*.[63] However, 54.8% (102/186) and 2.69% (5/186) general-EC isolated from diseased pigs in Spain had *mcr-4* and *mcr-5*, respectively.[69]

In ESBL-EC isolates tested from healthy pigs in UK (n=190) and Vietnam (n=39), none carried *mcr-1*.[50,62] In contrast, 31.2% (378/1213) isolates resistant to 3rd generation cephalosporins collected from healthy and diseased pigs in China also carried *mcr-1*.[64,65,70] In isolates collected from Germany which were either resistant to cefotaxime or enrofloxacin resistance, 10.6% (n=69/652) carried *mcr-1*.[55,71] Although none of the 214 ESBL-EC isolates collected from pigs at slaughter in Italy carried *mcr-2*, *mcr-3* or *mcr-5*, one of these isolates carried a *mcr-4* gene and 13 isolates carried *mcr-1* gene.[63] Additionally, 4 of the 9 carbapenem resistant isolates and 45 of the 101 enrofloxacin resistant isolates collected from China carried *mcr-1*.[61,72] Studies reporting prevalence of *mcr-1* in phenotypically colistin resistant isolates revealed that while only 13.2% of the Belgian isolates carried *mcr-1*, prevalences of this gene in colistin resistant isolates from China, France and Portugal were 96.3% (n=78/81), 88.6% (n=70/79) and 100% (n=90/90), respectively.[53,72–74]

Briefly, FQ-resistance was very low-low (<10%) in isolates from healthy animals collected from European countries, except for Iceland (moderate, 12.6%) and Romania,

Spain and Cyprus (high, between 29.3-43.7%) (Figure 2-9; table 2-11). Prevalences of NAL-resistance also ranged between very low- low (<10%) in European countries, except for moderate resistance (10.4-24.6%) for isolates collected from healthy animals in Croatia, Cyprus, Iceland, Romania and Spain (table 2-12). The prevalences of FQ- and NAL-resistance among PMP isolates recovered from Europe were also very low-low (<10%) except for isolates from Greece (FQ-resistance -15.8%) (tables 2-11 and 2-12).

The prevalences of FQ-resistance were higher in isolates cultured from diseased animals from European countries, with these prevalences ranging from low (Belgium, Czech Republic, Denmark, Norway, Sweden, United Kingdom) to moderate (Bulgaria, Finland, France, Switzerland, range-11.2 to 18.8%), high (Croatia, Estonia, Latvia, Lithuania, Portugal, Serbia and Spain, range-20.5 to 49.9%), and extremely high (Romania-78.3%) (figure 2-10; table 2-11). Similarly, except for isolates from Norway and United Kingdom, isolates from diseased animals in other European countries were moderately (Denmark-13.8%) to highly (Belgium, Croatia, Estonia, Finland, Latvia, Lithuania, Spain, Switzerland, range-20.8 to 47.2%) NAL-resistant (table 2-12). Notably, isolates from diseased animals in Portugal were extremely resistant to NAL (84.5%) (table 2-12).

For Asian countries, low prevalences of FQ- and NAL-resistance were rarely reported, with only isolates from healthy animals in Japan presenting low prevalences of resistance (0.96 -2.58%) (figure 2-9; tables 2-11 and 2-12). In isolates from healthy animals, prevalences of FQ- resistance ranged from moderate (South Korea-14.0%) to high (Cambodia, China, India, Thailand, Vietnam; range-24.3 to 47.3%) (figure 2-9; table 2-11). Prevalences of NAL-resistance were even higher in healthy isolates from these

countries, with prevalences ranging from high (Cambodia-20.6%; South Korea-36.9%) to extremely high (China, India, Thailand; range- 68.1 to 74.8%) (table 2-12).

Similarly, in Asian countries, isolates from diseased animals were consistently moderately to highly resistant against FQ and NAL (figure 2-10; tables 2-11 and 2-12). Except for isolates from India (9.40% FQ-resistance), isolates from Japan, Thailand and Vietnam were moderately FQ-resistant (10.4-15.4%) whereas isolates from South Korea (48.5%), China (67.2%) and Taiwan (85.2%) were high to extremely FQ-resistant (figure 2-10; table 2-11). Isolates from diseased animals tested for NAL-resistance were either highly resistant (Japan, India- 34.5 to 39.3%) or extremely resistant (China, South Korea, Taiwan- 55.9 to 95.1%) (table 2-12).

From certain other countries (Australia, Canada, Grenada, New Zealand, Nigeria, Uganda, United States of America), prevalences of FQ- and NAL-resistance in isolates collected from various sources were very low to low (<10%) except for moderate NAL-resistance in isolates from diseased pigs in Nigeria (15.5%) and high NAL-resistance in PMP isolates from United States of America (23.9%) (figures 2-19, 2-10; tables 2-11 and 2-12). Although prevalences of FQ-resistance in isolates from healthy animals in Brazil and Chile were low, isolates from diseased animals were moderately NA-resistant (Chile-11.8%) or highly FQ- and NAL-resistant (Brazil, 24.6-40.4%) (figures 2-9, 2-10; tables 2-11 and 2-12). Prevalence of resistance in isolates from Argentina varied from moderately FQ-resistant (healthy animals-11.9%), highly NAL-resistant (healthy animals-32.6%) and very highly FQ-resistant (diseased animals-57.1%) (figures 2-9, 2-10; tables 2-11 and 2-12). South African isolates from healthy animals were either moderately NAL-resistant (14.2) or highly FQ-resistant (21.7%) (figure 2-9; table 2-11

and 2-12). Isolates collected from PMP in Egypt were highly FQ-resistant (47.5%) (table 2-11).

Data on FQ- and NAL-resistance in extended spectrum beta-lactamase carrying *E. coli* (ESBL-EC, conferring resistance against 3rd and 4th generation cephalosporins) were available in 13 countries and are summarized in table 2-13. Except for Canada, where none of the 85 ESBL-EC isolates were ciprofloxacin resistant, percentage of ESBL-EC which were also ciprofloxacin resistant ranged from 12.8% (n=5/39) in Vietnam to 100% (n=54/54) in Taiwan (table 2-13). This phenomenon of co-resistance was also observed in ESBL-EC isolates collected from European countries such as Spain, Germany and Portugal. The percentages of ESBL-EC isolates resistant to NAL were even higher (table 2-13).

Genotypic information was available for *E. coli* isolates collected between 1993 and 2017 and described in 18 articles. None of the general-EC isolates recovered from healthy animals or PMP in Australia (n=72, healthy animals) and Czech Republic (n=27-healthy animals, 32-PMP) carried *qnrA*, *qnrB* or *qnrS* genes.[75–77] The percentage of *qnrA* in general-EC isolates from Asian countries ranged from zero (n=206 isolates-healthy animals, Thailand; 378 isolates- healthy animals, China) and 0.36% (n=6/1,679 isolates, diseased animals, China) to 11.1% (n=20/180, healthy animals, Vietnam).[78–87] The *qnrB* gene was not detected either among isolates from healthy animals in Thailand (n=206) and Vietnam (n=180) but was found at very low prevalences in healthy and diseased animals in China (1.88%, n=426 and 1.97%, n=1679 respectively). [78–87] Additionally, none of 1,545 and 305 isolates from diseased and healthy animals respectively in China carried the *qnrC* gene. [78–87] The *qnrD* gene was not detected in a collection of 1,469

isolates from diseased animals but was found in 11/378 (2.91%) isolates from healthy animals in China. [78–87] Finally, *qnrS* was the most prevalent group of PMQR genes in Asia, with 18.1% of the isolates from healthy animals (n=62/426-China, 78/180-Vietnam, 7/206-Thailand) and 12.3% of the isolates from diseased animals (n=196/1598, China) carrying this gene. [78–87]

In addition to the general-EC isolates, the presence of *qnr* genes has been also investigated in certain resistant isolates. In South Korea, 5.74% (10/174) of FQ-resistant isolates carried *qnrS* gene while *qnrA*, *qnrB* and *qnrD* were not present in these isolates.[88,89] In Denmark, none of the 39 FQ resistant isolates tested carried a *qnrA*, *qnrB* or *qnrS* genes.[90] In China, 15.2% (n=30/198) and 22.2% (n=8/36) of the ESBL-EC and *bla*$_{\text{CTX-M}}$ carrying isolates respectively also carried *qnrS* gene.[64,91] The *qnrD* gene was also present in 6 of these 36 *bla*$_{\text{CTX-M}}$ isolates, whereas none carried *qnrA*, *qnrB* or *qnrC* genes.[91]

Meta-analysis of factors affecting prevalences of antimicrobial resistance

Overall, the mixed-effects meta-analytic regression model estimated a 5–9% annual increase ($p<0.05$) in the odds of general-EC isolates resistant to ceftiofur, cefotaxime and ceftazidime as well as for ESBL-EC isolates, whereas an annual 2-3% decrease in odds ($p<0.05$) was found in the percentage of colistin, NAL- and FQ- resistance in general-EC (table 2-14).

In general, the odds of finding resistant general-EC were higher in isolates recovered from diseased pigs compared to healthy pigs/PMP: isolates recovered from diseased pigs were 1.4-6 times more likely to be resistant different antimicrobials compared with isolates from healthy pigs and porcine meat products (table 2-14). The

odds of ESBL-EC isolation were non-significantly higher among *E. coli* isolates recovered from diseased pigs as compared to healthy pigs and porcine meat products (table 2-14). General-EC isolates collected from LMIC were 3-11% more likely to be resistant to different antimicrobials under study ($p<0.01$) and 1.41 (95% CI-1.12-1.77) times more likely to be ESBL-C as compared to isolates collected from UIC (table 2-14).

Discussion

The WHO has classified colistin and 3rd, 4th, and 5th generation cephalosporins as critically important drugs for human medicine. Similarly, the OIE has classified cephalosporins (1st, 2nd and 3rd generation) as critically important, and colistin as highly important, for veterinary medicine. Emerging patterns of resistance to both of these antimicrobial classes are among the most concerning issues surrounding AMR in human medicine, better understanding of the relevant epidemiology in animal reservoirs is desirable. The aim of this systematic review and meta-analysis was to integrate available global information on the prevalence and temporal patterns of resistance to key antimicrobials in indicator bacteria collected from sources of swine origin. In general, the prevalences of cephalosporin and colistin resistance were higher among isolates from LMIC countries compared to UIC countries. Prevalences of resistance were also higher among isolates collected from diseased pigs compared to isolates from healthy pigs or porcine meat products, except for prevalence of ESBL-EC. A wide repertoire of genes encoding resistance to cephalosporins, colistin and carbapenems have been identified among swine *E. coli* isolates.

The range of cephalosporins used in food-producing animals is narrower than in human medicine. First- and second-generation cephalosporins have been widely available

for animal use since the 1970s, and some later generation cephalosporins were developed strictly for veterinary use. Ceftiofur (3rd generation) is approved for use in many countries, while a fourth-generation cephalosporin (cefquinome) has been approved in the European Union and some other countries. In general, prevalences of cephalosporin resistance were higher in LMIC, which could be a consequence of the unregulated use of cephalosporins.[92] Acknowledging the importance of cephalosporin resistance in human and veterinary medicine, preservation of this class of antimicrobials is a topic of debate among policy makers.[93] The observed reduction in cephalosporin resistance in Danish isolates of food animal origin after the enforcement of a voluntary ban is an example of how policy making can mitigate the threat of extended spectrum cephalosporin resistance.[94] Other measures that may mitigate cephalosporin resistance in animals include using third and fourth generation cephalosporins as second line of treatment and permitting their use only if indicated by antimicrobial susceptibility testing.[95]

β-lactamases, including ESBLs, are the most important resistant mechanisms in Enterobacteriaceae and their corresponding encoding genes (*bla* genes) have served as a basis for their classification.[96] Certain alleles of specific ESBLs families, including *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{CTX-M}}$, have been described as particularly successful.[97] Moreover, *bla*$_{\text{CTX-M}}$ family of genes can be sub-classified into specific allele types and the distribution of these alleles provide useful context to molecular epidemiology of ESBL resistance. Another common but non-ESBL genetic mechanism for third generation cephalosporin resistance are *bla*$_{\text{CMY}}$ family and its alleles, which are plasmid mediated AmpC-β-lactamases.[98]

Genes belonging to the *bla*$_{TEM}$ family were found to be widespread in isolates collected from various swine sources. Widespread distribution of *bla*$_{TEM}$ genes among *Enterobacteriaceae* was already reported as early as 1970s, a concerning finding given that they can confer resistance to both penicillins and cephalosporins.[99] However, *bla*$_{TEM-1}$, the most prevalent specific allele belonging to this *bla*$_{TEM}$ gene family, confers resistance to penicillins but not extended spectrum cephalosporins.[99] Hence, the high prevalence of these genes alone in general-EC and ESBL-EC populations is not necessarily indicative of widespread 3rd and 4th generation cephalosporin resistance.

bla$_{SHV}$ genes have been cited to be widely displaced by the emergence of *bla*$_{CTX-M}$ and *bla*$_{TEM}$ genes in both human and animal populations.[100] In this systematic review, we also observed a comparatively low prevalence of *bla*$_{SHV}$ genes in ESBL-EC isolates collected from swine sources worldwide, with the exception of a few countries. However, unlike any of the *bla*$_{CTX-M}$ or *bla*$_{TEM}$ alleles, a *bla*$_{SHV}$ allele (*bla*$_{SHV}$-38) has been able to confer resistance to carbapenems.[101] Hence, the relatively lower prevalence of *bla*$_{SHV}$ genes should not understate their importance in conferring extended cephalosporin resistance.

bla$_{CTX-M}$ are a diverse group of genes that have undergone a rapid diversification and global dissemination post-2000 and are considered to be the dominant genetic mechanism behind the ESBL phenotype.[102] Mirroring our results in swine, another systematic review found similar *bla*$_{CTX-M}$ genes to be globally prevalent in human isolates.[103] However, these parallelisms in global distribution of *bla*$_{CTX-M}$ genes should not imply that swine are a major source of these genes for humans, as recent systematic

reviews and research have found the directionality within these potential relationships to be unclear.[104,105]

The global spread of bacteria resistant to third and fourth generation cephalosporins has led to an increased consumption of last resort antimicrobials such as carbapenems in human medicine,[106] which may lead to selection of other resistances such as those mediated by carbapenemases. Carbapenemases are β-lactamases capable of hydrolyzing not only carbapenems but also penicillins and third generation cephalosporins, and can be transmitted between different bacterial species via plasmids.[107] It should be noted that carbapenems are not licensed for use in food in any country and hence, the risk factors behind the presence of carbapenem resistant in pigs or porcine meat products needs to be further evaluated.[106] Although the phenotypic and genotypic prevalence of carbapenem resistance is extremely low, the significance of these results should not be understated. The widespread dissemination of carbapenem resistance is a relatively recent phenomenon as evidenced by the post-2010 changes in the extent and prevalence of carbapenem resistance in bacterial populations in European countries.[108] Considering the mechanistic similarities in propagation and dissemination of extended spectrum cephalosporin and carbapenem resistance (such as plasmid mediated carriage of genetic elements, broad range substrate specificity, broad bacterial host range, etc.), carbapenem resistance could become established in bacterial populations as has been the case with resistance to extended spectrum cephalosporin in *E. coli* from swine. Carbapenem resistance has already become endemic in certain settings, such as the invasive bacterial isolates collected from diseased human populations in Greece and

Italy,[108] and therefore, there is an active need for assessing the risk of food borne sources such as swine products as a source of carbapenem resistance.[109]

Colistin is considered a last resort antibiotic for treatment of Gram-negative bacterial infections in human medicine.[110] Colistin is widely used as a growth promotant in countries like India, and Vietnam, in addition as a treatment for digestive disorders in pigs.[111] As a result, even low or moderate prevalences of phenotypic resistance in *E. coli* collected from swine sources should be a matter of significant concern. Previously, colistin resistance was thought to spread slowly via vertical transmission and with a slow pace of evolution, as this resistance was considered unstable and due to chromosomal mutations.[14] However, recognition worldwide of plasmid mediated genes encoding colistin resistance has ignited the concerns in relation to several bacterial pathogens of humans.[112] Our systematic review clearly documents the global distribution of plasmid mediated mechanism of colistin resistance in swine populations and porcine meat products. Readers are further referred to a review article wherein the authors discuss the public health implications, the pharmacology and use of colistin in pigs.[113]

Since their discovery in the early 1960s, quinolones (such as nalidixic acid) and fluoroquinolones (such as ciprofloxacin) have played a critical role in both human and veterinary medicine.[114] Fluoroquinolones are amongst the most commonly prescribed antimicrobials for treating urinary tract infections in humans[115] and are also used worldwide for treatment for diseases in food animals such as bovine and respiratory diseased complex.[116] In general, the prevalences of FQ-resistance were low in isolates from European and North American countries. However, there were certain exceptions, as evidenced by the high prevalences of FQ- and NAL-resistance in Spain and Portugal.

The prevalences of FQ- and NAL- resistance described here roughly correlate with consumption of FQs for those countries in which data was available: for example, the prevalences of FQ-resistance were higher in healthy and diseased animals from Spain and Portugal compared to countries such as Netherlands, Denmark and Sweden, in which the mg per population correction unit consumption of FQs is also lower.[117] Similarly, the prevalence of FQ- and NAL- resistance was consistently low in Australia, a country where use of these antimicrobials has been banned in farm animals since before 2000.[118] This correlation between AMR and antimicrobial use in food animals at the country level has been already reported for FQ and other antimicrobial classes[119] but should be interpreted with care due to the risk of ecological fallacies when analyzing grouped data.

In comparison to chromosomal mutations and efflux pump activity, PMQRs are a recent discovery, with the first true PMQR element (a *qnr* gene) being found in a human patient in 1998.[36] Nevertheless, since then *qnr* genes have been found in plasmids varying in size and incompatibility specificity.[36] *qnr* genes, transposable elements, plasmid incompatibility types and geographic spread have been recently reviewed in in detail by Hooper and Jacoby et al. (2015),[35] that described the presence of *qnr* genes in bacteria of different origins in a far larger number of countries compared to those in present study. Hence, *qnr* genes might be present but undetected in swine populations or food products. Hence, more studies are needed to conclusively determine the spread of *qnr* genes in swine sources across several countries.

Co-resistance against multiple drug classes makes the problem of antimicrobial resistance in bacteria even more complex, as use of one class of antimicrobials can provide positive selection pressure to maintain resistance against other antimicrobial

class.[13] Quinolone and extended spectrum cephalosporin co-resistance has been reported in Gram-negative isolates collected from various sources in Canada, India and Italy.[120–122] Results from the current study also suggest that co-selection between FQ, NAL and extended spectrum cephalosporins is occurring at a varying level in swine isolates from various countries.

Across the studies we reviewed, clinical isolates were more resistant to quinolones, cephalosporins and colistin compared to isolates from healthy animals and porcine meat product. Some authors have suggested that treatment of diseased pigs before sample collection may lead to higher prevalences of AMR compared to healthy populations.[95] Although AMR in clinical isolates is likely not representative of AMR in isolates from healthy swine populations, surveillance of AMR in clinical isolates can still be useful for monitoring purposes. In the absence of organized active surveillance programs, clinical submissions can be used as a passive surveillance system for monitoring levels of AMR, particularly against therapeutic agents such as quinolones, cephalosporins and colistin. This approach can be particularly useful in resource limited settings where diagnostic laboratories can play dual roles by enabling some AMR surveillance from routine diagnostic cases, although submission bias must be heeded. Importantly, clinical isolates can act as an early indicator of emerging resistance phenotypic and genotypic patterns.[9]

The pattern of higher prevalences of AMR in LMIC may be associated with the high levels of antimicrobial use in swine production in these countries.[23] This problem may increase if, antimicrobial usage in food animal production increases in the upcoming decades in developing countries as projected.[23,123] To date, the focus of AMR

surveillance in developing countries has been on human health, and AMR surveillance in food animal production has been largely ignored.[124] It is advised that LMIC include food animal populations (including swine) in their AMR surveillance programs.

Several limitations of this study must be borne in mind when interpreting the results. First, only articles written in English were included in the systematic literature review, thus potentially leading to the exclusion of data from many countries. Similarly, when estimating the changes of resistance over time and across geographical regions the different breakpoints (CLSI vs EUCAST epidemiological cut-offs) and laboratory methods (diffusion vs dilution methods, pooled vs non-pooled samples) used were not considered in the analysis due to the lack of consistent information. More robust estimates could be obtained using MIC or inhibition diameters for comparison of the resistance prevalences from different studies/regions/periods, but unfortunately the majority of the eligible articles reviewed did not provide the adequate quantitative information (distribution of MIC/inhibition diameters).

Because the purpose of this study was to obtain estimates on the frequency of resistance to cephalosporins, carbapenems and colistin in *E. coli* of swine origin, articles focusing on single new/unusual genes, description of single plasmids or case reports and articles reporting data from a single farm/outbreak were not included. As a result, the reports of specific genes encoding resistance to the antimicrobials of interest of this review may have been missed if they were only reported very occasionally. Also, as reflected by the wide confidence intervals, there was considerable uncertainty around the estimates from certain countries/region/antimicrobials/sources where sample sizes were smaller and data from different research studies was pooled (particularly LMIC). In

contrast, estimates from countries with an AMR surveillance program on *E. coli* of animal origin (e.g. European countries reporting to EFSA, Japan, South Korea or Canada) and larger sample sizes had less associated statistical uncertainty. Furthermore, the representativeness of the *E. coli* populations included in research-based studies will be typically lower than that of systematic surveillance programs, which underlines the importance of conducting guided surveillance programs to accurately estimate prevalence of antimicrobial resistance.

Conclusions

This study summarizes the existing evidence on prevalence of resistance to certain critical antimicrobials in *E. coli* isolates collected from various swine sources and describes some differences between periods and regions. The literature showed a moderate increase in antimicrobial resistance to selected critical antimicrobials over time, as well as differences in resistance levels in isolates collected from countries of different economic status, and across different sources of bacterial isolation. Genotypic resistance to critical antimicrobials was found to be prevalent globally. Although at a low level, carbapenem resistance was observed globally both phenotypically and genotypically. We hope that this review will serve as a useful baseline for both scientists and policy makers in understanding the current status of antimicrobial resistance in swine production globally.

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Tables

Table 2-1. Characteristics of articles included in the literature review

Characteristic of articles	Sub-categories	Number of articles (%)
A) Continent from where isolates were recovered (n=324 articles)	Europe	158 (48.8%)
	Asia	101 (31.2%)
	North America	43 (13.3%)
	South America	9 (2.77%)
	Africa	6 (1.85%)
	Australia	7 (2.16%)
B) Year of publication (n=324 articles)	1983-1999	8 (2.47%)
	2000-2005	49 (15.1%)
	2006-2010	68 (21.0%)
	2011-2015	113 (34.9%)
	2016- July 2018	86 (26.5%)
C) Source of isolates (n=324 articles)	Diseased pigs	117 (36.1%)
	Healthy pigs	154 (47.5%)
	Mixed (healthy and diseased) pigs	12 (3.70%)
	Clinical status not defined	61 (18.8%)
	Porcine meat products	62 (19.1%)
	Diffusion	124 (41.3%)

D) Laboratory methods used in articles with phenotypic data (n=300 articles)	Dilution	173 (57.7%)
	VITEK	2 (0.67%)
	Method not clear	2 (0.67%)
E) Interpretive criteria used for classification of cephalosporin resistance (n=266 articles)	CLSI	163 (61.3%)
	EUCAST	53 (19.9%)
	Others	11 (3.39%)
	None	40 (15.0%)
F) Articles with genotypic information (n=109)	Cephalosporin resistance	79 (72.5%)
	Colistin resistance	28 (25.7%)
	Carbapenem resistance	13 (11.9%)

Table 2-2. Prevalence of ceftiofur resistance in general-EC isolates across different sources

Country	Healthy pigs	Diseased pigs	PMP	References
Argentina	6.10 (0-20.3) [21]	23.9 (13.5-36.3) [54]	NA	[125,126]
Australia	2.03 (0.01-6.21) [598]	21.02(0.14-59.54) [286]	NA	[77,127–131]
Austria	0(0-0.35) [744]		NA	[132]
Belgium	2.53(1.49-3.77) [921]	1.26(0.1-3.24) [298]	NA	[133,134]
Brazil	2.5(0.77-4.94) [267]	NA	NA	[45]
Canada	0.58(0.22-1.05) [16911]	12.03(3.01-25.4) [1309]	1.21(0.5-2.13) [2977]	[135–161]
China	21.1 (12.3-31.4) [8469]	43 (31.2-55.1) [1072]	NA	[54,64,72,86,91,162–168]
Czech Republic	NA	3.97(1.51-7.33) [217]	NA	[169]

Denmark	0.31 (0.08-0.64) [3709]	1.15(0.41-2.15) [1450]	0.57(0.06-1.4) [941]	[26,57,132,170–176,176–192]
Estonia	4.24(1.33-8.38) [179]	3.09(0.31-7.81) [94]	NA	[132,191,193]
Finland	0.01(0-0.67) [526]	1.03(0-3.19) [258]	NA	[191,194–197]
France	0.53(0-3.07) [126]	2.6 (2.36-2.85) [20072]	NA	[191,198]
Germany	2.65(0-9.01) [53]	NA	NA	[191,199]
Greece	1.15(0-4.5) [165]	NA	NA	[200,201]
Iceland	0.69(0-3.64) [109]	NA	1.14(0-6.53) [50]	[202]
Ireland	0.61(0.08-1.45) [802]	NA	NA	[11]
Italy	0.16(0-1.44) [269]	NA	NA	[134]
Japan	0.3(0-1.09) [1191]	NA	NA	[203–206]
South Korea	NA	3.87(1-8.13) [575]	NA	[207–210]
Lithuania	0.86(0-5.37) [60]	3.39(0.08-9.84) [60]	NA	[211]

Norway	0.11(0-0.9) [510]	1.04(0-3.62) [177]	0.23(0-1.69) [234]	[191,212–214]
Poland	1.4(0-7.24) [692]	NA	NA	[134,215]
Romania	NA	22.34(0-71.81) [32]	NA	[216,217]
Serbia	NA	5.33(1.11-11.89) [74]	NA	[218]
Spain	0.01(0-0.44) [757]	11.25(2.01-25.84) [332]	NA	[134,219]
Sweden	0(0-0.68) [284]	0.1(0-0.4) [3892]	NA	[191,220,220–226]
Switzerland	1.05(0-3.36) [188]	0.39(0-3.32) [92]	NA	[134,191]
Taiwan	17.51(0-72.86) [711]	NA	NA	[227]
Thailand	3.07(0-14.11) [23]	8.86(0-34.35) [170]	NA	[228]
USA	5.34(0.02-16.52) [4273]	8.29(4.48-13.03) [5159]	14.52(3.75-30.18) [120]	[229–237]
Vietnam	NA	2.49(0-11.98) [187]	NA	[238,239]

Numbers outside bracket represent pooled percent prevalence of resistance. Numbers in round brackets represent 95% confidence interval of the estimate. Numbers in squared brackets represent the number of isolates tested.

Table 2-3. Prevalence of cefotaxime resistance in general-EC isolates across different sources

Country	Healthy pigs	Diseased pigs	PMP	References
Australia	NA	0.66(0-4.54) [70]	NA	[240]
Austria	0.76(0.16-1.64) [1012]	NA	NA	[41,132,191,241–244]
Belgium	2.23(0.62-4.53) [548]	NA	NA	[41,241,245]
Bulgaria	0.59(0.06-1.45) [1024]	NA	NA	[41,246–249]
Cambodia	1.49 (0.07-4.08) [261]	NA	NA	[250]
Canada	NA	2.57(0.21-6.68) [112]	NA	[138]
Chile	0.32(0-3.01) [100]	NA	NA	[251]

China	12.7 (1.29-32.3) [1274]	18.5 (4.47-38.8) [1614]	NA	54,64,67,86,91,163,252–256
Croatia	0.46(0-3.64) [85]	2.02(0-8.8) [370]	NA	41,257,258
Cuba	NA	2.67(0-12.57) [26]	NA	259
Cyprus	5.57(0.8-13.46) [55]	NA	NA	41
Czech Republic	2.22 (0.06-6.39) [214]	NA	3.75(0-1.348) [32]	41,75,76
Denmark	0.57(0.16-1.13) [2046]	2.08(0.56-4.28) [311]	0.6(0-0.226) [258]	26,41,132,183–186,186–188,190,192,241–244,260–263
Estonia	5.67(1.97-10.85) [414]	3.47(0.48-8.39) [94]	NA	41,191,191,193,242–244,264
Finland	0.3(0-1.28) [1448]	4.06(2.08-6.56) [749]	NA	41,191,196,197,243,265–267
France	0.66(0.21-1.28) [1556]	NA	NA	132,191,241–244,244,260,261,263
Germany	7.99(0-27) [1051]	NA	2.43(0-0.934) [46]	41,242,260,261,268,269
Greece	4.93(1.61-9.64) [159]	NA	NA	41,200

Grenada	0.04(0-1.41) [180]	NA	NA	[270]
Hungary	1.11(0-3.41) [238]	NA	NA	[41,241]
Iceland	0.69(0-3.64) [109]	NA	2.19(0-0.857) [50]	[202]
India	28.21(12.04-47.85) [376]	35.72(5.21-74.85) [419]	NA	[271–278]
Ireland	0.38(0-2.54) [147]	NA	NA	[41]
Italy	0.25(0-1.09) [567]	NA	NA	[41,132,263]
Japan	1.32(0-7.29) [45]	NA	2.79(0-1.304) [25]	[279]
South Korea	0.37(0-4.1) [4381]	1.23(0-4.26) [130]	NA	[209,280–282]
Latvia	0.09(0-1.81) [150]	NA	NA	[41]
Lithuania	0.42(0-3.45) [89]	NA	NA	[41]
Malaysia	9.86(0-37.93) [7]	NA	NA	[283]
Malta	0.7(0-4.69) [68]	NA	NA	[41]

Netherlands	0.32(0.08-0.66) [4309]	NA	2.27(0.81-0.426) [981]	[41,132,191,241–244,260–262,284–294]
Nigeria	73.72(65.4-81.28) [121]	NA		[295]
Norway	0.77(0-3.11) [918]	NA	2.55(0-1.212) [27]	[41,132,191,242,296]
Poland	1.6(0.79-2.61) [1426]	NA	NA	[41,241,242,244,297]
Portugal	2.34(0-7.36) [264]	NA	NA	[41,298]
Romania	0.97(0.09-2.42) [399]	NA	NA	[41]
Slovakia	0.46(0-3.64) [85]	NA	NA	[41]
Slovenia	0.46(0-3.64) [85]	NA	NA	[41]
South Africa	15.8 (4.97-30.8) [169]	NA	NA	[299]
Spain	0.44(0.11-0.91) [2387]	NA	NA	[41,132,191,242,244,260–262,300–302]

Sweden	0.17(0-0.71) [1452]	0.44(0-1.99) [269]	NA	[27,41,132,191,221,242,262,263,303]
Switzerland	0.28(0-0.93) [902]	0.27 (0-2.54) [119]	NA	[41,241–244,304]
Taiwan	2.5(0.06-7.2) [360]	NA	NA	[227]
Thailand	32.7 (0-86.5) [724]	10.4(5.58-16.37) [131]	NA	[48,78,228,305,306]
UK	0.05(0-1.52) [170]	0.16(0-1.64) [205]	1.16(0-0.525) [79]	[41,307,308]

Numbers outside bracket represent pooled percent prevalence of resistance. Numbers in round brackets represent 95% confidence interval of the estimate. Numbers in squared brackets represent the number of isolates tested.

Table 2-4. Prevalence of ceftazidime resistance in general-EC isolates across different sources

Country	Ceftazidime resistance in healthy pigs	Ceftazidime resistance in diseased pigs	Ceftazidime resistance in food products	References
Argentina	3.51 (0-15.4) [21]	NA	NA	[126]

Australia	1.78 (0.46-3.70) [325]	0.73(0-3.57) [114]	NA	[127,128]
Austria	0.75(0-2.56) [303]	NA	NA	[41,241]
Belgium	1.95(0.74-3.57) [548]	NA	NA	[41,241,245]
Bulgaria	0.69(0.11-1.56) [1024]	NA	NA	[41,246–249]
Cambodia	2.82 (0.10-8.0) [261]	NA	NA	[250]
China	2.89(0.33-7.25) [997]	12.5 (0.68-34.07) [509]	NA	[54,56,67,86,91,163,252,309]
Croatia	0.55(0-3.76) [85]	NA	NA	[41]
Cyprus	5.69(0.91-13.56) [55]	NA	NA	[41]
Czech Republic	1.3(0.03-3.72) [187]	NA	NA	[41]
Denmark	0.24(0-1.08) [528]	NA	0.71(0-4.45) [73]	[41,188,190]

Egypt	NA	NA	62.66(49.93-74.57) [59]	[310]
Estonia	5.28(1.69-10.42) [218]	NA	NA	[41,242,243,264]
Finland	0.03(0-1.18) [217]	NA	NA	[41]
France	0.7(0.18-1.46) [1023]	NA	NA	[41,191,241–244]
Germany	0.91(0-3.75) [991]	NA	2.55(0-9.45) [46]	[41,242,269,311]
Greece	3.82(0.97-8.09) [159]	NA	NA	[41,200]
Grenada	0.08(0-1.52) [180]	NA	NA	[270]
Hungary	2.89(0-9.44) [183]	NA	NA	[41]
India	NA	99.9(98.36-100) [170]	NA	[274]
Ireland	0.47(0-2.65) [147]	NA	NA	[41]
Italy	0.27(0-1.46) [317]	NA	NA	[41,191]
South Korea	0.77(0-3.58) [4013]	3.11(0.25-8.2) [204]	NA	[209,280,312–314]
Latvia	0.15(0-1.92) [150]	NA	NA	[41]

Lithuania	0.51(0-3.57) [89]	NA	NA	[41]
Malaysia	9.97(0-37.96) [7]	NA	NA	[283]
Malta	0.8(0-4.81) [68]	NA	NA	[41]
Netherlands	0.42(0.08-0.94) [2379]	NA	1.96(0.55-3.98) [861]	[41,191,241–243,284–290,290]
Nigeria	74.48(66.26-81.93) [121]	13.03(0-47.46) [5]	NA	[295,315]
Norway	0.17(0-1.03) [462]	NA	NA	[41,242]
Poland	1.12(0.34-2.2) [1242]	NA	NA	[41,241,242,297]
Portugal	2.46(0.02-7.48) [264]	NA	NA	[41,298]
Romania	1.08(0.16-2.54) [399]	NA	NA	[41]
Slovakia	0.55(0-3.76) [85]	NA	NA	[41]
Slovenia	0.55(0-3.76) [85]	NA	NA	[41]

South Africa	13.3 (3.36-28.0) [169]	NA	NA	[299]
Spain	0.48(0.06-1.17) [1174]	NA	NA	[41,242,244,300–302]
Sweden	0.05(0-1.32) [200]	NA	NA	[41]
Switzerland	0.44(0.03-1.14) [902]	0.27 (0-2.54) [119]	NA	[41,241–244,304]
Taiwan	0.39(0-1.58) [360]	1.8(0-7.06) [61]	NA	[227,316]
Thailand	18.5 (0.52-51.21) [724]	3.61(0.89-7.71) [131]	NA	[48,78,228,305,306]
UK	0 (0-0.38) [2650]	NA	NA	[41,317]
Vietnam	3.16(0-13.05) [323]	0.25(0-2.38) [126]	NA	[79,239,318]

Numbers outside bracket represent pooled percent prevalence of resistance. Numbers in round brackets represent 95% confidence interval of the estimate. Numbers in squared brackets represent the number of isolates tested.

Table 2-5. Prevalence of phenotypic ESBL resistance in general-EC isolates across different sources

Country	Healthy pigs	Diseased pigs	PMP	References
Austria	1.46(0.02-4.22) [163]	NA	NA	[41]
Belgium	0.21(0-1.87) [186]	9.86(0-37.93) [7]	NA	[41]
Brazil	2.5(0.77-4.94) [267]	NA	NA	[45]
Bulgaria	3.4(0-15.35) [21]	NA	NA	[41]
Cambodia	10.86(3.39-21.49) [47]	NA	NA	[319]
China	16.98(2.58-39.51) [794]	43.14(38.58-47.77) [458]	NA	[64,163,252,320–323]
Croatia	0.46(0-3.64) [85]	NA	NA	[41]
Cyprus	5.57(0.8-13.46) [55]	NA	NA	[41]
Czech Republic	4.41(0-18.21) [353]	NA	2.08(0-10.28) [32]	[41,76,324]

Denmark	4.21(1.89-7.26) [4684]	NA	0.56(0.11-1.21) [1213]	26,41,94,185–188,325
Estonia	1.04(0-4.84) [85]	NA	NA	41
Finland	0.13(0-0.67) [1374]	NA	0(0-0.61) [303]	41,265,267
France	0.17(0-1.7) [200]	NA	NA	41
Germany	3.44(1.23-6.5) [212]	2.12(0.56-4.37) [2187]	NA	41,326–328
Greece	4.89(1.48-9.82) [116]	NA	NA	41
Hong Kong	79.34(72.86-85.17) [172]	NA	NA	329
Hungary	0.8(0-3.11) [170]	NA	NA	41
India	16.3 (7.25-28) [1045]	25.07(18.74-31.96) [170]	NA	274,275,330,331
Ireland	0.38(0-2.54) [147]	NA	NA	41
Italy	4.92(0-27.89) [268]	NA	NA	41,332

Japan	1.76 (0.01-5.4) [1225]	NA	2.79(0-13.04) [25]	[51,279,333,334]
South Korea	18.8 (9.76-29.8) [59]	1.95 (0.1-5.31) [585]	NA	[209,335,336]
Latvia	0.09(0-1.81) [150]	NA	NA	[41]
Lithuania	0.42(0-3.45) [89]	NA	NA	[41]
Malaysia	9.86(0-37.93) [7]	NA	NA	[283]
Malta	0.7(0-4.69) [68]	NA	NA	[41]
Netherlands	13.1 (7.64-19.8) [2713]	NA	0.3(0-0.91) [1538]	[41,284–286,337]
Nigeria	31.42(27.7-35.25) [600]	NA	NA	[338]
Norway	0.27(0-0.91) [905]	NA	NA	[41,339–341]
Poland	3.49(0.02-10.71) [422]	NA	NA	[41,342,343]
Portugal	15.54(0-48.53) [317]	9.85(0-31.21) [13]	NA	[41,344–346]

Romania	0.97(0.09-2.42) [399]	NA	NA	[41]
Slovakia	0.46(0-3.64) [85]	NA	NA	[41]
Slovenia	0.46(0-3.64) [85]	NA	NA	[41]
Spain	27.07(0-99.19) [199]	NA	NA	[41]
Sweden	0.46(0-1.47) [1139]	NA	0.2(0-1.21) [486]	[27,41]
Switzerland	7.58 (2.84-14.1) [354]	NA	1.14(0-6.53) [50]	[41,347–351]
Taiwan	NA	19.29(14.74-24.26) [275]	NA	[352]
Thailand	58 (28-85.1) [664]	11.93(6.79-18.19) [131]	NA	[48,52,228,319,353]
UK	7.53(0-42.62) [807]	NA	2.42(0.01-7.37) [79]	[41,307,354]

Vietnam	19.1 (0-87) [163]	NA	31.82(24.44-39.67) [147]	[50,318,355]

Numbers outside bracket represent pooled percent prevalence of resistance. Numbers in round brackets represent 95% confidence interval of the estimate. Numbers in squared brackets represent the number of isolates tested.

Table 2-6. Pooled estimates of percentage prevalence of *bla* and *mcr-1* genes in general-EC populations

Country	*bla*$_{SHV}$	*bla*$_{CTX-M}$	*bla*$_{CMY}$	*bla*$_{TEM}$	*mcr-1*	References
Australia	3.72(0-14.3) [142]	0.73(0-3.18) [142]	0.75(0-4.64) [70]	54.9(26.5-81.8) [142]	NA	[77,240]
Belgium	NA	NA	NA	NA	0.41 (0-3.13) [100]	[356]
Bulgaria	NA	NA	NA	4.78(1.91-8.69) [186]	NA	[248]

Canada	0.81(0-3.53) [166]	NA	2.54(0.29-6.32) [166]	5.33(0-27.7) [166]	NA	[147,149]
China	0.37(0-1.29) [675]	1.53(0-5.29) [724]	NA	36.9(3.5-80.3) [681]	14.1 (6.56-23.7) [1140]	[14,56,67,82,86,252,320,357,358]
Czech Republic	2.43(0-8.14) [59]	2.43(0-8.14) [59]	NA	17.5(0-65.4) [59]	NA	[75,76]
Denmark	NA	3.17(0.97-6.33) [1691]	0.76(0.07-1.92) [668]	22.9(9.12-40.3) [27]	0 (0-0.11) [689]	[26,185,187,359]
France	NA	NA	NA	NA	0 (0-0.24) [1458]	[356,360]

Germany	NA	2.37(0.27-5.92) [1649]	NA	NA	0.56 (0-1.84) [2388]	[326,327,356,361]
Hungary	5.71(0-23.5) [13]	5.71(0-23.5) [13]	NA	90.0(68.7-99.9) [13]	1.67 (0-8.34) [40]	[356,362]
India	0 (0.-0.04) [867]	0.09(0.27-1.79) [867]	4.35(3.01-5.9) [867]	2.96(1.84-4.30) [867]	NA	[331]
Italy	NA	NA	NA	NA	0.36 (0-2.26) [168]	[63]
Japan	NA	NA	NA	NA	4.93 (0-22.87) [2736]	[68,363]

Netherlands	NA	5.88 (0.04-18.2) [2236]	0.17(0-0.65) [1779]	NA	0 (0-0.11) [3489]	[284–286,356]
Poland	0(0-0.6) [337]	NA	0(0-0.6) [337]	49.6(44.2-54.9) [337]	NA	[215]
Portugal	NA	7.73(2.94-14.3)	NA	NA	NA	[74]
South Korea	0.01(0-0.95) [249]	0.13(0-1.36) [249]	NA	NA	0.19 (0-1.60) [220]	[335,364]
Spain	NA	NA	NA	NA	6.02 (0-19.48) [1298]	[69,356,365]

Sweden	0.12(0-0.68) [1375]	0.66(0.11-1.50) [1675]	0.15(0-0.72) [1375]	NA	NA	[303]
Switzerland	NA	7.75(0.7-20.3) [29]	NA	NA	NA	[351]
Thailand	0.05(0-1.27) [206]	NA	0.05(0-1.27) [206]	94.2(77.3-100) [550]	NA	[78,366]
UK	NA	3.79(0.48-9.4) [79]	NA	NA	0.01 (0-0.91) [258]	[307,356]
USA	NA	0.57(0-1.74) [1694]	38.3(11.9-69.0) [2194]	33.4(13.8-56.5) [199]	0.04 (0-0.63) [563]	[58,367,368]

Venezuela	NA	NA	NA	NA	2.08 (0.01-6.3) [93]	[369]
Vietnam	NA	NA	NA	NA	18.7 (13.2-24.8) [180]	[79]

Numbers outside bracket represent pooled percent prevalence of resistance. Numbers in round brackets represent 95% confidence interval of the estimate. Numbers in squared brackets represent the number of isolates tested.

Table 2-7. Pooled estimates of percentage prevalence of select *bla* genes in ESBL-EC isolates and isolates resistant against extended spectrum cephalosporins

Country	*bla*$_{SHV}$	*bla*$_{CTX-M}$	*bla*$_{CMY}$	*bla*$_{TEM}$	References
Belgium	15.5 (11.4-20.1) [278]	87.7(83.5-91.4) [278]	NAs	35.5(29.9-41.3) [278]	[370]
Brazil	NA	91.1(65.5-100) [8]	NA	NA	[45]
Cambodia	13.0 (0-47.5) [5]	13.0(0-47.5) [5]	NA	NA	[319]
Canada*	0.54 (0-3.75) [85]	3.49(0.41-8.71) [85]	96.5(91.3-99.6) [85]	49.4(38.8-60.1) [85]	[371]
China	1.14 (0-3.85) [266]	76.5(42.0-98.2) [556]	2.82(0.41-0.68) [203]	NA	[54,64,163,253,323,372,373]

Czech Republic	7.16(0-23.4) [18]	92.8(76.7-100) [18]	NA	7.16(0-23.4) [18]	[324]
Denmark	15.3(0-54.1) [4]	29.2(0.71-74.3) [4]	15.3(0-54.1) [4]	NA	[325]
Finland	8.89(0-34.5) [8]	8.89(0-34.5) [8]	8.89(0-34.5) [8]	8.89(0-34.5) [8]	[267]
Germany	1.43(0-5.91) [72]	94.6(89.9-97.9) [253]	1.75(0-5.46) [106]	18.7(0-68.9) [178]	[47,328,374,375]
Hong Kong	NA	42.2(32.0-52.7) [368]	NA	NA	[329]
India	45.0(16.1-75.9) [12]	51.1(17.4-84.3) [63]	NA	31.7(17.6-47.8) [63]	[275,330,376]

Italy	NA	65.9(41.0-86.9) [15]	NA	NA	[332]
Japan	NA	96.6(85.2-100) [22]	NA	NA	[51]
Netherlands	NA	99.9(99.1-100) [259]	NA	NA	[337]
South Korea	62.1(52.1-71.7) [95]	62.3 (3-100) [104]	13.0(2.85-28.4) [107]	99.6(96.7-100) [95]	[49,209,336]
Poland	NA	64.6(26.8-94.2) [6]	35.4(5.79-73.2) [6]	NA	[343]
Portugal	23.5(0-74.2) [2]	64.2(18.5-97.9) [38]	NA	23.5(0-74.2) [2]	[298,344,345]
Spain	33.7(17.3-52.3) [27]	52.7(14.1-89.5) [56]	2.66(0-12.2) [27]	2.66(0-12.2) [27]	[377,378]

Switzerland	NA	52.7(0-100) [29]	63.1(14.2-98.9) [4]	NA	[348,350]
Taiwan	3.33(0-14.8) [22]	92.1(84.0-97.7) [76]	23.6(8.44-43.1) [22]	54.4(33.8-74.3) [22]	[352,379]
Thailand	3.64(0-12.3) [64]	68.4(16.7-100) [64]	NA	NA	[48,319]
UK	2.22(0-19.2) [2629]	94.2(89.7-97.6) [149]	NA	0.32(0.07-0.68) [2480]	[317,354]
Vietnam	1.40(0-5.14) [92]	85.7(66.7-97.7) [131]	NA	66.5(44.0-85.7) [131]	[50,355,380]

*- This data is based on isolates resistant against extended spectrum cephalosporins (ceftiofur)

Numbers outside bracket represent pooled percent prevalence of resistance. Numbers in round brackets represent 95% confidence interval of the estimate. Numbers in squared brackets represent the number of isolates tested

Table 2-8. Global percent prevalence of specific *bla*$_{\text{CTX-M}}$ alleles in ESBL-EC isolates

bla$_{\text{CTX-M}}$ alleles	Global percent prevalence of specific alleles (number of isolates with specific allele/total number of isolates)	Countries from where the presence of specific alleles have been reported	Prevalence of specific alleles in Asian countries among ESBL-EC (number of isolates with specific allele/total number of isolates)	Prevalence of specific alleles in European countries among ESBL-EC (number of isolates with specific allele/total number of isolates)
bla$_{\text{CTX-M-1}}$	67.3 (602/895)	China, Denmark, Finland, Germany, Hong Kong, India, Italy, Netherlands, Portugal, Spain,	43.2 (80/185)	73.5 (522/710)

		Switzerland, United Kingdom		
bla$_{\text{CTX-M-2}}$	2.70 (7/259)	Netherlands	-	2.70 (7/259)
bla$_{\text{CTX-M-3}}$	1.41 (8/568)	China, Germany, Japan, Switzerland, United Kingdom	1.29 (5/387)	1.66 (3/181)
bla$_{\text{CTX-M-9}}$	32.5 (126/388)	Hong Kong, India, Portugal, Spain	47.7 (104/218)	12.9(22/170)
bla$_{\text{CTX-M-14}}$	16.6 (148/893)	China, Germany, Japan, Netherlands, Portugal, South Korea, Spain,	29.2 (102/349)	8.46 (46/544)

		Switzerland, United Kingdom		
bla$_{\text{CTX-M-15}}$	12.4 (139/1122)	Brazil, Cambodia, China, Germany, Italy, Japan, Netherlands, South Korea, Taiwan, Thailand, United Kingdom	15.6 (91/585)	7.56 (40/529)
bla$_{\text{CTX-M-22}}$	2.99 (5/167)	China	2.99 (5/167)	NA
bla$_{\text{CTX-M-24}}$	0.67 (1/149)	China, Germany	1.16 (1/86)	0 (0/63)
bla$_{\text{CTX-M-27}}$	5.19 (18/347)	China, United Kingdom	7.58 (15/198)	2.01 (3/149)

bla$_{\text{CTX-M-28, 36, 69}}$	0 (0/167)	China	0 (0/167)	NA
bla$_{\text{CTX-M-32}}$	1.35 (6/443)	Netherlands, Portugal, United Kingdom	NA	1.35 (6/443)
bla$_{\text{CTX-M-55}}$	1.44 (90/624)	China, Japan, Taiwan, United Kingdom	18.5 (88/475)	1.34 (2/149)
bla$_{\text{CTX-M-64}}$	2.02 (4/198)	China	2.02 (4/198)	NA
bla$_{\text{CTX-M-65}}$	12.1 (46/381)	China, South Korea, Taiwan	12.1 (46/381)	NA
bla$_{\text{CTX-M-97}}$	12.7 (8/63)	Germany	NA	12.7 (8/63)
bla$_{\text{CTX-M-104}}$	2.02 (4/198)	China	2.02 (4/198)	NA
bla$_{\text{CTX-M-123}}$	3.03 (6/198)	China	3.03 (6/198)	NA
bla$_{\text{CTX-M-125}}$	1.52 (3/198)	China	1.52 (3/198)	NA

Table 2-9. Global prevalence of carbapenem resistance in general-EC isolates

Country	Number of isolates tested for carbapenem resistance	Number of isolates resistant against carbapenem	References
Australia	509	2	127,128,240
Austria	163	0	41
Belgium	186	0	41
Bulgaria	21	0	41
Cambodia	261	0	250
China	805	11	54,60,61,67,163,253,254
Croatia	85	0	41
Cyprus	55	0	41
Czech Republic	187	0	41
Denmark	2050	0	26,41,187,188

Estonia	85	0	[41]
Finland	1146	0	[41,265]
France	279	0	[41]
Germany	661	4	[41,55]
Greece	167	6	[41,381]
Grenada	180	0	[270]
Hungary	170	0	[41]
India	112	23	[382]
Ireland	147	0	[41]
Italy	168	0	[41]
Japan	92	0	[279]
South Africa	169	3	[299]
South Korea	599	10	[207,312]
Spain	186	0	[41,69,301,302]
Switzerland	119	0	[41,304]

Latvia	150	0	[41]
Lithuania	89	0	[41]
Malaysia	7	0	[283]
Malta	68	0	[41]
Netherlands	2159	3	[41,284–286,291–294]
Norway	270	0	[41]
Poland	170	0	[41]
Portugal	264	1	[41,298]
Romania	399	0	[41]
Slovakia	85	0	[41]
Slovenia	85	0	[41]
Sweden	200	0	[41]
Thailand	654	2	[78,353]
UK	249	0	[41,307]

Table 2-10. Prevalence of colistin resistance in general-EC isolates across different sources

Country	Healthy pigs	Diseased pigs	PMP	References
Australia	0.04 (0-0.91) [325]	NA	NA	[127]
Austria	0.3(0-2.2) [163]	NA	NA	[41]
Belgium	0.28 (0-1.28) [443]	9.21(5.02-14.39) [157]	NA	[41,245,383]
Brazil	NA	22.7 (10.1-38.4) [300]	NA	[384–387]
Bulgaria	3.4(0-15.35) [21]	5.91(3.01-9.6) [220]	NA	[41,388]
Cambodia	16.3 (4.27-33.6) [261]	NA	NA	[250]
China	28.9 (21-37.3) [10500]	4.55(0-27.9) [1018]	NA	[64,65,72,168,256]
Croatia	0.46(0-3.6) [85]	2.1(0.62-4.2) [370]	NA	[41,257,258]
Cyprus	0.98(0-5.9) [55]	NA	NA	[41]
Czech Republic	1.02 (0-4.81) [214]	NA	2.08(0-10.28) [32]	[41,75,76]

Denmark	0.1 (0.01-0.29) [6025]	0.75(0.24-1.45) [1835]	0.61(0.13-1.31) [1200]	26,41,170–190,260,261,389
Estonia	1.43(0-4.37) [156]	NA	NA	41,264
Finland	0(0-0.32) [782]	2.84(0.08-8.1) [76]	NA	41,265,266
France	0.15(0-0.57) [6193]	NA	NA	41,260,261,360,390
Germany	1.1 (0.32-2.21) [2853]	NA	NA	41,260,261,361
Greece	0.72(0-3.4) [159]	NA	NA	41,200
Hungary	0.71(0-3.26) [210]	NA	NA	41
Ireland	0.1(0-1.86) [147]	NA	NA	41
Italy	1.67 (0-6.13) [550]	NA	NA	41,63
Japan	0.38(0.01-1.1) [1375]	34.36(3.37-76.08) [802]	NA	203–206,363,391–393
South Korea	0.81(0.32-1.45) [3514]	17.43(2.81-40.01) [664]	NA	207,208,364,394
Laos	23.23(7-44.94) [18]	NA	NA	395

Latvia	0.36(0-2.5) [150]	NA	NA	[41]
Lithuania	1.42(0-4.36) [149]	1.72(0-7.07) [60]	NA	[41,211]
Malaysia	9.86(0-37.93) [7]	NA	NA	[283]
Malta	0.7(0-4.69) [68]	NA	NA	[41]
Netherlands	0.02(0-0.32) [2023]	NA	1.16(0-3.88) [519]	[260,261,284–288]
Norway	0(0-0.75) [270]	NA	NA	[41]
Poland	0.05(0-1.52) [170]	NA	NA	[41]
Portugal	2.09(0.34-4.83) [198]	NA	NA	[41]
Romania	0.09(0-0.97) [399]	NA	NA	[41,216]
Serbia	NA	33.76(23.35-45) [74]	NA	[218]
Slovakia	0.46(0-3.64) [85]	NA	NA	[41]
Slovenia	0.46(0-3.64) [85]	NA	NA	[41]
Spain	1.2 (0.18-2.90) [2176]	24.72(1.19-63.0) [272]	2.05 (0-7.87) [55]	[41,69,219,260,261,300,365,396]

Sweden	0.03(0-0.84) [367]	1.39(0.05-3.84) [269]	3.59(0-16.06) [20]	27,41,220,221,303

Numbers outside bracket represent pooled percent prevalence of resistance. Numbers in round brackets represent 95% confidence interval of the estimate. Numbers in squared brackets represent the number of isolates tested.

Table 2-11. Prevalence of fluoroquinolone (FQ) resistance in general-E isolates across different sources

Country	Healthy pigs	Diseased pigs	PMP	References
Argentina	11.9 (6.26-19.0) [104]	57.1 (43.7-70.1) [54]	NA	125,126,397,398
Australia	1.37 (0.40-2.73) [535]	0.09 (0-1.16) [295]	NA	127,128,130,131,240,399
Austria	3.13 (2.08-4.34) [1526]	NA	NA	41,132,191,241–244
Belgium	5.41 (0.89 -12.7) [1615]	4.01 (0.73-9.20) [431]	NA	41,133,134,241,245
Brazil	0 (0-0.87) [141]	40.4 (24.2-57.8) [153]	NA	385,386,400

Bulgaria	5.37 (1.62 -10.8) [1024]	18.8 (13.8-24.3) [220]	NA	[41,246–249,388]
Cambodia	40.6 (5.98 -81.9) [261]	NA	NA	[250]
Canada	0 (0-0) [21420]	6.16 (0-22.1) [510]	0 (0-0.30) [2977]	[135,136,139,140,142–146,148–161,401,402]
Chile	7.71 (3.93-12.5) [187]	NA	NA	[251,403]
China	47.3 (37.2-57.6) [10139]	67.2 (55.5-77.9) [2568]	NA	[54,56,64,72,84–87,91,162–168,252,254,256,309,404,405]
Croatia	6.89 (2.25-13.5) [85]	21.9 (15.7-28.9) [370]	NA	[41,257,258]
Cuba	NA	0.32 (0-7.38) [26]	NA	[259]
Cyprus	43.7 (30.8-57.0) [55]	NA	NA	[41]
Czech Republic	1.47 (0.10-3.83) [214]	3.61 (0.37-9.16) [80]	6.85 (0.47-18.4) [32]	[41,75,76,169]
Denmark	0.16 (0 -0.84) [5935]	2.48 (0.94-4.55) [4721]	0.31 (0.01-0.91) [1078]	[26,41,57,132,170,172–191,241–244,260–263,389,406]

Egypt	NA	NA	47.5 (34.8-60.3) [59]	310
Estonia	7.16 (0.9 -17.4) [403]	20.5 (1.03-53.4) [94]	NA	41,132,191,193,242–244,264
Finland	0.77 (0.19-1.62) [917]	12.6 (6.74-19.7) [1364]	NA	194–197,265,266,407
France	3.66 (1.97 -5.76) [1856]	11.2 (10.7-11.6) [19471]	NA	41,132,191,198,241–244,260,261,263
Germany	1.71 (0 -6.11) [1382]	NA	6.76 (1.06-16.1) [46]	41,191,199,242,260,261,269,311
Greece	5.81 (1.35-12.6) [281]	NA	15.8 (7.0-27.2) [51]	41,200,201,381
Grenada	0 (0-0.56) [180]	NA	NA	270
Hungary	9.57 (5.82-16.3) [238]	NA	NA	41,241
Iceland	12.6 (6.87-19.7) [109]	NA	8.16 (1.95-17.6) [50]	202

India	27.9 (13.7-44.9) [393]	9.40 (6.78-12.4) [454]	NA	271,272,276–278,408–410
Ireland	2.09 (0.55 -4.32) [949]	NA	NA	11,41
Italy	6.86 (1.99 -14.0) [836]	NA	NA	41,134,191,263
Japan	0.96 (0 -2.96) [1359]	10.4 (0.15-30.6) [118]	20.7 (7.17-38.5) [25]	203–206,279,392,411
Latvia	4.95 (1.83-9.23) [150]	22.4 (9.51-38.7) [31]	NA	41,134
Lithuania	5.30 (0.05-16.0) [149]	23.6 (7.19-45.3) [109]	NA	41,211,412,413
Malaysia	17.8 (0.15-51.5) [7]	NA	NA	283
Malta	5.84 (1.25-12.9) [68]	NA	NA	41
Netherlands	8.22 (0.29-23.4) [5914]	NA	2.13 (1.20-3.26) [981]	41,132,191,241–244,260–262,284–294,414,415
New Zealand	0 (0-0.16) [296]	NA	NA	416
Nigeria	7.13 (3.02-12.6) [121]	96.3 (65.7-100) [5]	NA	295,315
Norway	5.67 (0-34.0) [655]	0 (0-1.18) [177]	0 (0-0.29) [419]	191,212–214,243,296

Poland	9.07 (6.44-12.1) [2118]	NA	NA	41,134,215,241,242,244,297
Portugal	8.97 (0-34.9) [264]	49.9 (25.1-74.8) [122]	NA	41,134,298
Romania	29.3 (24.9-33.9) [399]	78.3 (63.8-89.9) [38]	NA	41,216,217
Serbia	NA	25.7 (16.8-35.8) [82]	NA	218,417
Slovakia	6.89 (2.25-13.5) [85]	NA	NA	41
Slovenia	5.01 (0.06-15.1) [115]	NA	NA	41
South Africa	21.7 (2.88 -50.4) [169]	NA	NA	299
South Korea	14.0 (7.50-22.0) [4556]	48.5 (27.5-69.9) [731]	NA	88,207,208,210,280–282,312–314,394
Spain	33.3 (11.7-59.3) [3200]	42.4 (20.0-66.6) [672]	0 (0-0.89) [180]	41,69,132,134,191,219,242,244,260–262,300–302,418,419
Sweden	3.65 (0.24-9.75) [1385]	5.70 (4.66-6.83) [5977]	3.25 (0-15.8) [20]	27,41,132,220–226,262,263,303,415
Switzerland	3.61 (2.44-4.97) [1102]	15.1 (0-59.7) [384]	NA	41,134,191,241–244,304,420,421

Taiwan	NA	85.2 (75.1-93.2) [61]	NA	[316]
Thailand	48.7 (21.1-76.7) [1372]	15.4 (0-81.8) [251]	NA	[48,78,228,305,306,366,422,423]
Uganda	NA	0 (0-1.47) [100]	NA	[424]
United Kingdom	1.93 (0.19-4.87) [170]	3.64 (1.17-7.14) [1235]	NA	[41,134,308]
United States of America	0 (0-0) 1963]	0 (0-0) [5159]	6.25 (1.58 -13.3) [120]	[229–231,233–237]
Vietnam	24.3 (10.5-41.4) [443]	14.6 (0-69.0) [187]	16.1 (3.38-35.1) [20]	[79,238,239,318,425]

Numbers outside bracket represent pooled percent prevalence of resistance. Numbers in round brackets represent 95% confidence interval of the estimate. Numbers in squared brackets represent the number of isolates tested.

Table 2-12. Prevalence of nalidixic acid (NAL) resistance in general-EC isolates across different sources

Country	Healthy pigs	Diseased pigs	PMP	References
Argentina	32.6 (22.9-43.1) [83]	NA	NA	[397,398]
Australia	1.87 (0-20.8) [9]	2.77 (0.06-7.99) [77]	0 (0-1.07) [142]	[240,399,426]
Austria	2.49 (1.65-3.46) [1756]	NA	2.35 (0.42-5.38) [179]	[41,132,191,241–244,427]
Belgium	6.11 (2.42-11.1) [1615]	24.9 (18.2-32.3) [431]	NA	[41,133,134,241,245]
Brazil	24.6 (17.7-32.2) [141]	NA	NA	[400]
Bulgaria	24.6 (8.79-44.8) [21]	NA	NA	[41]
Cambodia	20.6 (11.1-31.9) [261]	NA	NA	[250]
Canada	0 (0-0) [20230]	0.38 (0-2.51) [150]	0.05 (0-0.33) [3165]	[142–146,148–161,402,428]
Chile	11.8 (6.03-19.0) [100]	NA	NA	[251]

China	68.1 (41.2-89.9) [1056]	91.9 (75.3-100) [1243]	NA	64,85,91,162,167,252,254,309,404,405
Croatia	10.4 (4.64-18.0) [85]	31.0 (25.4-36.9) [256]	NA	41,257
Cuba	NA	12.3 (2.41-27.7) [26]	NA	259
Cyprus	21.9 (11.9-33.9) [55]	NA	NA	41
Czech Republic	0.61 (0-2.48) [24]	NA	9.95 (1.82-22.8) [32]	41,75,76
Denmark	0.26 (0.05-0.58) [5385]	13.8 (9.59-18.6) [2294]	1.57 (0.12-4.09) [993]	26,41,132,171–191,241–244,263,389,429,430
Estonia	3.32 (1.66 -5.43) [414]	30.8 (17.3-46.2) [94]	NA	41,132,191,193,242–244,264
Finland	2.13 (0.72 -4.08) [1207]	20.8 (14.6-27.6) [493]	NA	41,191,194–197,243,265,266
France	3.0 (2.14-3.96) [1654]	NA	NA	41,132,191,241–244,263
Germany	6.90 (0.01-21.7) [401]	NA	6.76 (1.06-16.1) [46]	41,191,242,269

Greece	7.22 (3.94 -11.3) [281]	NA	NA	[41,200,201]
Hungary	5.55 (2.57 -9.42) [693]	NA	NA	[41,132,241]
Iceland	12.6 (6.87-19.7) [109]	NA	8.16 (1.95 -17.6) [50]	[202]
India	74.8 (47.3-94.6) [387]	39.3 (3.49-84.0) [540]	NA	[271,272,276–278,409,410]
Ireland	1.66 (0.04-4.73) [147]	NA	NA	[41]
Italy	7.16 (3.18-12.4) [836]	NA	NA	[41,134,191,263]
Japan	2.58 (1.24-4.29) [1961]	34.5 (22.5-47.6) [118]	NA	[203–206,391–393,411]
Latvia	3.61 (0.98 -7.46) [150]	35.2 (19.5-52.8) [31]	NA	[41,134]
Lithuania	7.68 (2.84-14.4) [89]	35.0 (22.3-48.8) [49]	NA	[41,412,413]
Malaysia	97.5 (74.1-100) [7]	NA	NA	[283]
Malta	4.36 (0.52-10.8) [68]	NA	NA	[41]
Netherlands	0.72 (0.13-1.61) [3566]	NA	2.41 (1.11-4.08) [874]	[41,132,191,241–244,284–292]

Nigeria	1.78 (0-12.7) [231]	15.5 (0-53.3) [57]	NA	[295,431]
Norway	0 (0-0.23) [1043]	0.20 (0 -1.91) [177]	0 (0-0.48) [579]	[41,132,191,212–214,242,296]
Poland	6.05 (4.70-7.53) [2202]	NA	NA	[41,132,134,215,241,242,244,297]
Portugal	8.76 (5.51-12.6) [264]	84.5 (70.2-94.9) [33]	NA	[41,134,298]
Romania	15.6 (12.1-19.4) [399]	NA	NA	[41]
Slovakia	6.89 (2.25-13.5) [85]	NA	NA	[41]
Slovenia	8.04 (0-50.4) [115]	NA	NA	[41]
South Africa	14.2 (2.83-31.6) [169]	NA	NA	[299]
South Korea	36.9 (21.2-54.0) [3876]	55.9 (25.0-84.5) [160]	NA	[207,208,280]
Spain	18.1 (14.5-22.1) [2907]	47.2 (32.8-62.0) [586]	NA	[41,69,132,134,191,242,244,300–302]

Sweden	0.58 (0.06-1.42) [1248]	NA	0.56 (0-9.67) [20]	41,132,191,220,242,263
Switzerland	3.23 (2.14-4.50) [1090]	25.9 (19.8-32.4) [245]	NA	41,191,241–244,304,421
Taiwan	NA	95.1 (87.9-99.4) [61]	NA	316
Thailand	70.8 (30.7-97.9) [576]	NA	NA	48,305,422
Uganda	NA	8.77 (3.82-15.3) [100]	NA	424
United Kingdom	0.03 (0-0.20) [3748]	2.37 (0-14.4) [205]	NA	41,308,317,432–435
United States of America	1.45 (0-5.26) [1963]	0 (0-1.71) [230]	23.9 (4.04 -52.6) [120]	229,230,233–237
Vietnam	NA	NA	30.6 (12.7-52.1) [20]	425
Zambia	0 (0-0.72) [157]	NA	NA	436

Numbers outside bracket represent pooled percent prevalence of resistance. Numbers in round brackets represent 95% confidence interval of the estimate. Numbers in squared brackets represent the number of isolates tested.

Table 2-13. Percentage prevalence of fluoroquinolone (FQ) and nalidixic acid (NAL) resistance in ESBL-EC populations

Country	FQ-resistance	NA-resistance	Reference
Brazil	100 (8)	NA	[45]
Canada	0 (85)	0 (85)	[371]
China	63.2 (429)	82.4 (318)	[64,321–323,372]
Germany	28.7 (286)	42.7 (75)	[46,47,326]
Hong Kong	27.2 (206)	68.4 (206)	[329]
Japan	13.6 (22)	40.9 (22)	[51]
South Korea	77.8 (9)	88.9 (9)	[336]
Portugal	17.1 (35)	28.6 (35)	[346]
Spain	30.3 (66)	54.5 (66)	[377,437]

Switzerland	0 (9)	66.7 (9)	[368]
Taiwan	100 (54)	94.4 (54)	[352]
Thailand	44.4 (54)	52.2 (134)	[48,52]
Vietnam	12.8 (39)	23.1 (39)	[50]

Numbers outside bracket represent raw percentage of ciprofloxacin or NA- resistance in ESBL-EC isolates. Numbers inside the round brackets represent the number of ESBL-EC isolates tested for ciprofloxacin and NA-resistance.

Table 2-14. Changes in prevalence of AMR based on the random-effects model including time (years), source of isolates, and income levels of countries as fixed effects and country as a random effect

Antimicrobial/ ESBL-EC	**Odds ratio**	**p-value**
a) Cefotaxime		
Time	1.09 (1.07-1.12)	<0.01
Diseased (reference=Healthy/PMP)	1.76 (1.48 -1.09)	<0.01
LMIC (reference=UMIC)	4.88 (1.66 -14.35)	<0.01
b) Ceftiofur		
Time	1.05 (1.03-1.06)	<0.01
Diseased (reference=Healthy/PMP)	4.25 (3.80-4.77)	<0.01
LMIC (reference=UMIC)	3.58 (1.28-10.03)	0.015
c) ESBL-EC		
Time	1.05 (1.03 -1.08)	<0.01
Diseased (reference=Healthy/PMP)	1.04 (0.85-1.26)	0.71
LMIC (reference=UMIC)	1.41 (1.12-1.77)	<0.01
d) Ceftazidime		
Time	1.09 (1.05 -1.13)	<0.01
Diseased (reference=Healthy/PMP)	1.35 (0.94-1.94)	0.10
LMIC (reference=UMIC)	11.44 (3.76-34.72)	<0.01
e) Colistin		
Time	0.97 (0.96-0.98)	<0.01

Diseased (reference=Healthy/PMP)	5.96 (5.02 -7.08)	<0.01
LMIC (reference=UMIC)	5.97 (2.25-15.91)	<0.01
f) NAL-resistance		
Time	0.98 (0.97-0.99)	<0.001
Diseased (reference=Healthy/PMP)	4.49 (4.10-4.93)	<0.001
LMIC (reference=UMIC)	3.58 (1.52-8.46)	0.004
g) FQ-resistance		
Time	0.98 (0.97-0.99)	<0.001
Diseased (reference=Healthy/PMP)	1.60 (1.50-1.70)	<0.001
LMIC (reference=UMIC)	2.99 (1.49-5.99)	0.002

Figures

Figure 2-1. Literature search flowchart with application of inclusion/exclusion criteria

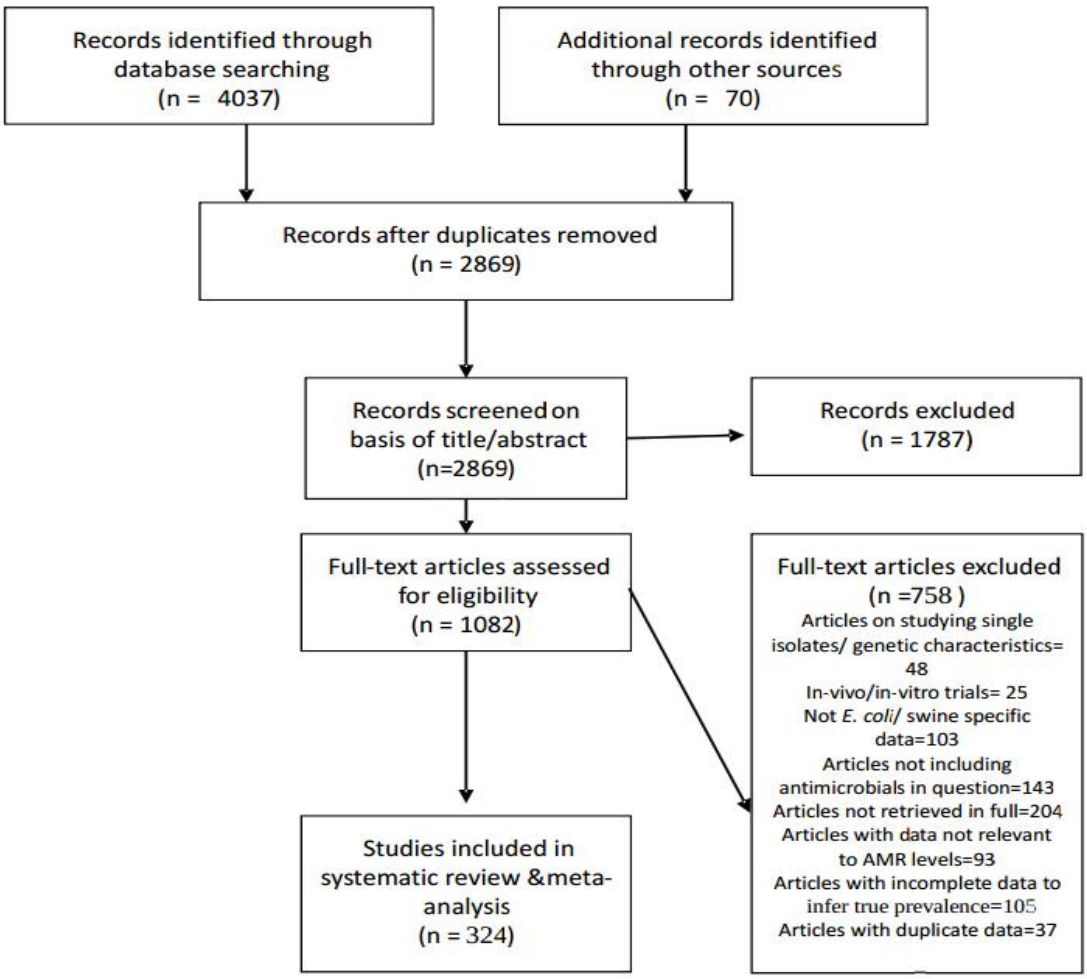


Figure 2-2. Global prevalence of ceftiofur resistance in general-EC collected from healthy pigs

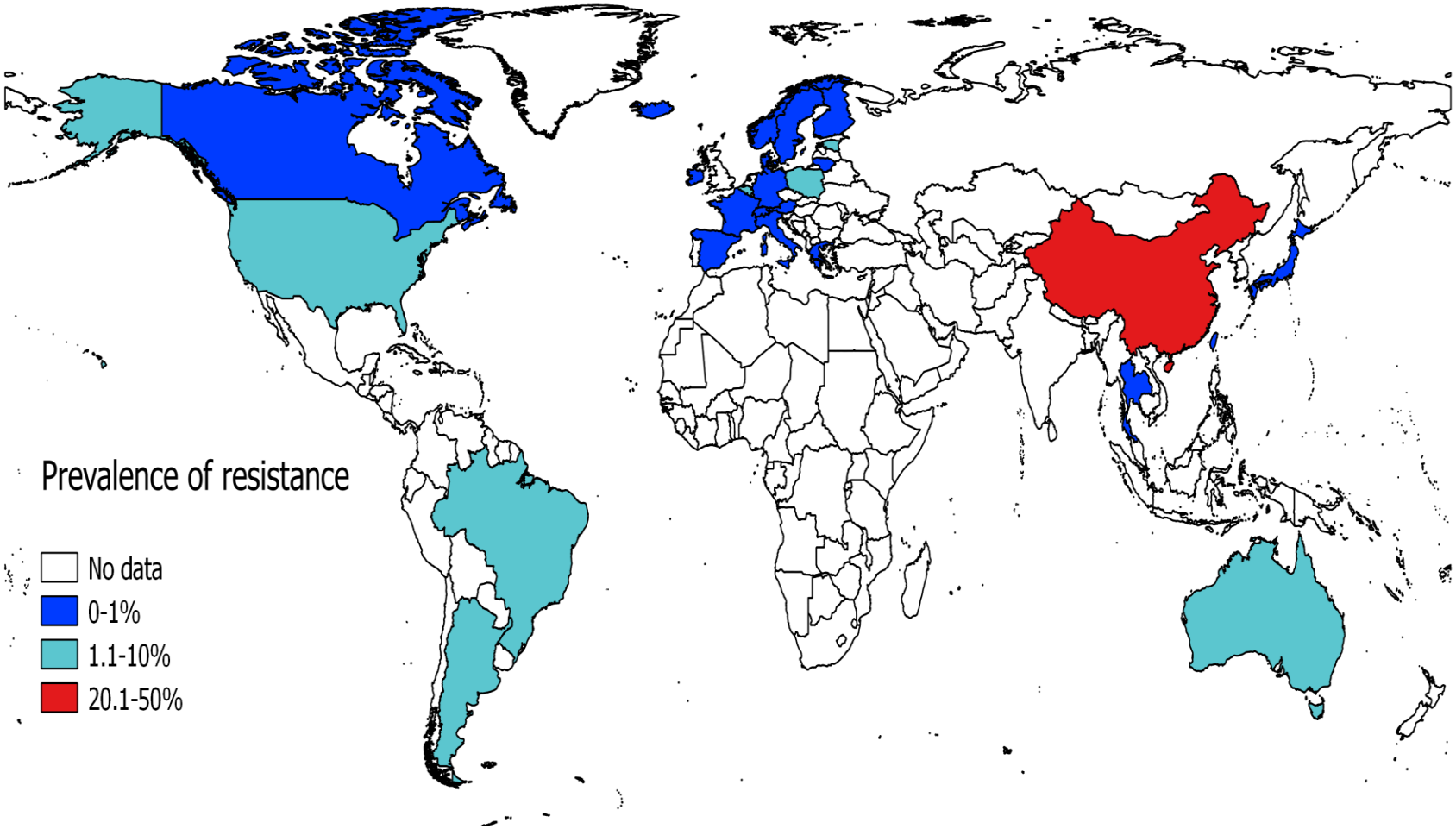


Figure 2-3. Global prevalence of ceftiofur resistance in general-EC collected from diseased pigs

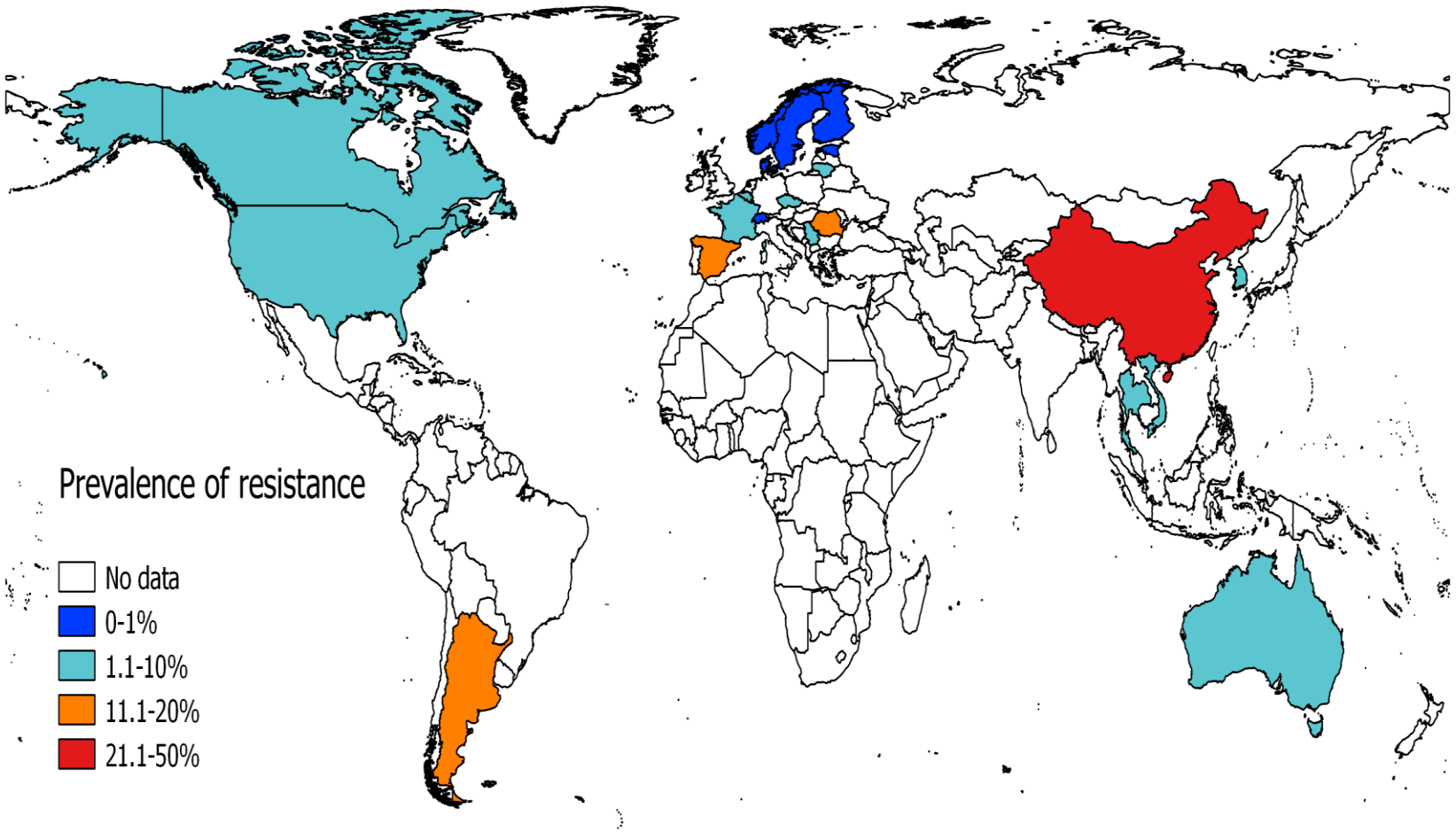


Figure 2-4. Global prevalence of cefotaxime resistance in general-EC collected from healthy pigs

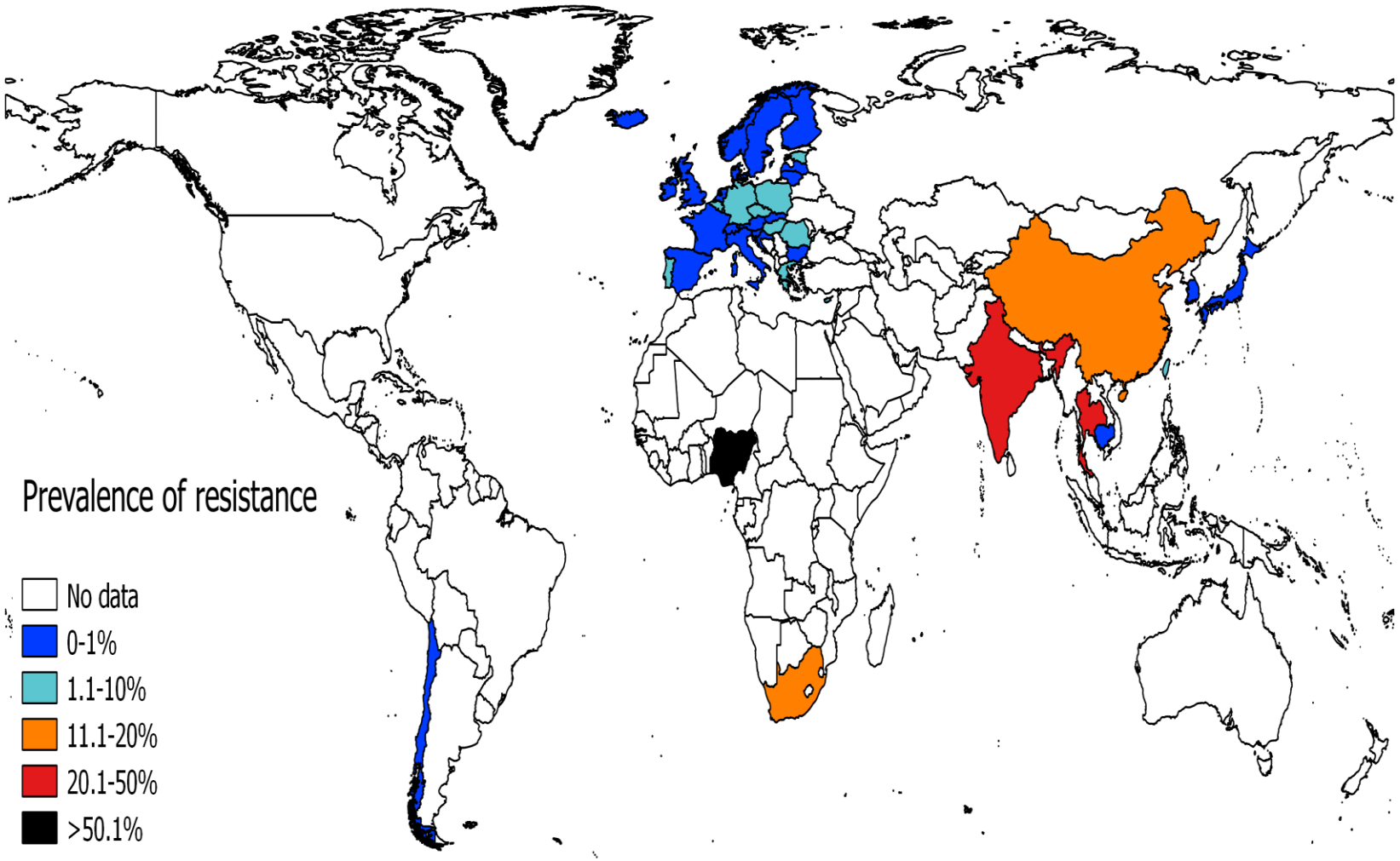


Figure 2-5. Global prevalence of cefotaxime resistance in general-EC from diseased pigs

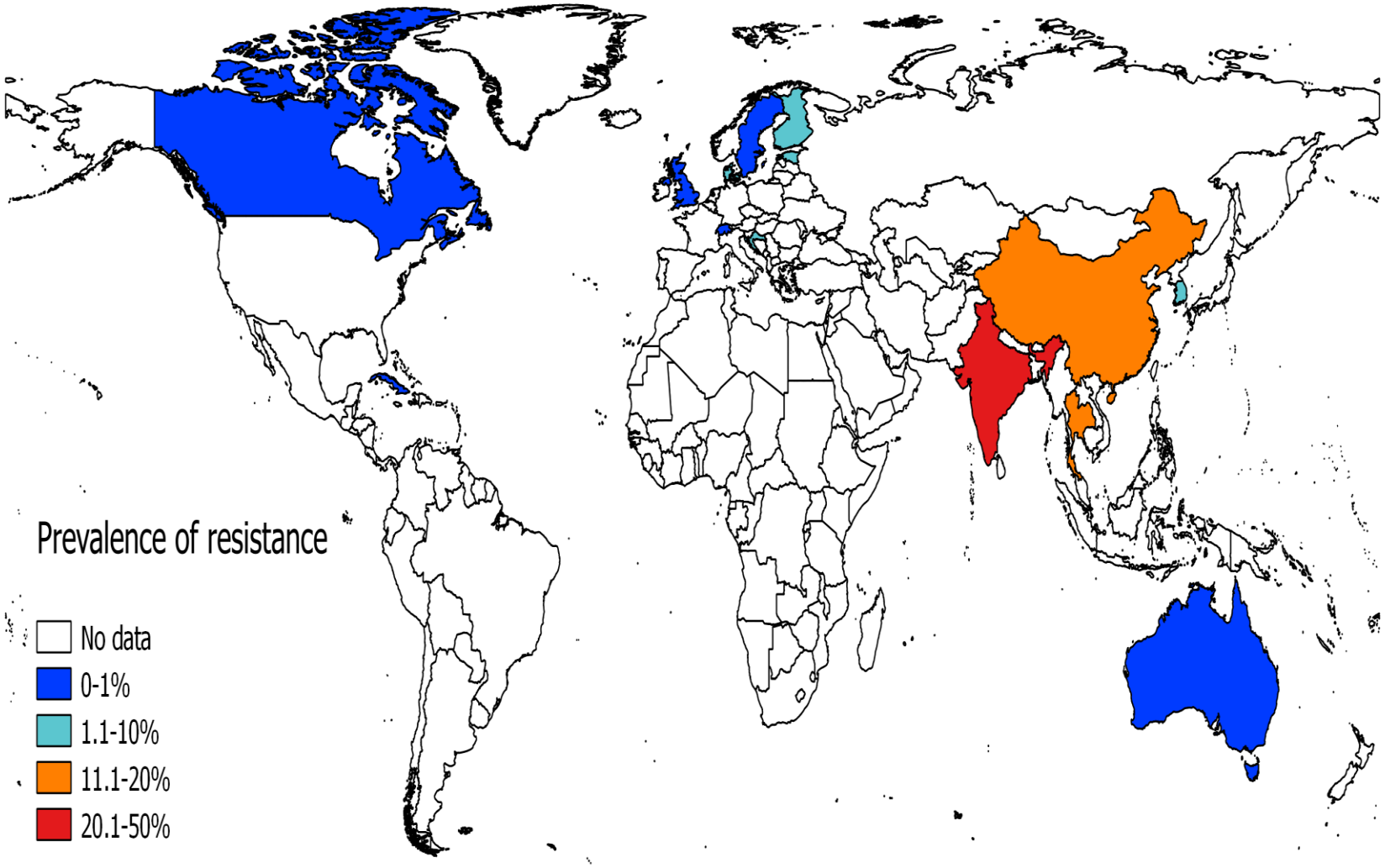


Figure 2-6. Global prevalence of ceftazidime resistance in general-EC from healthy pigs

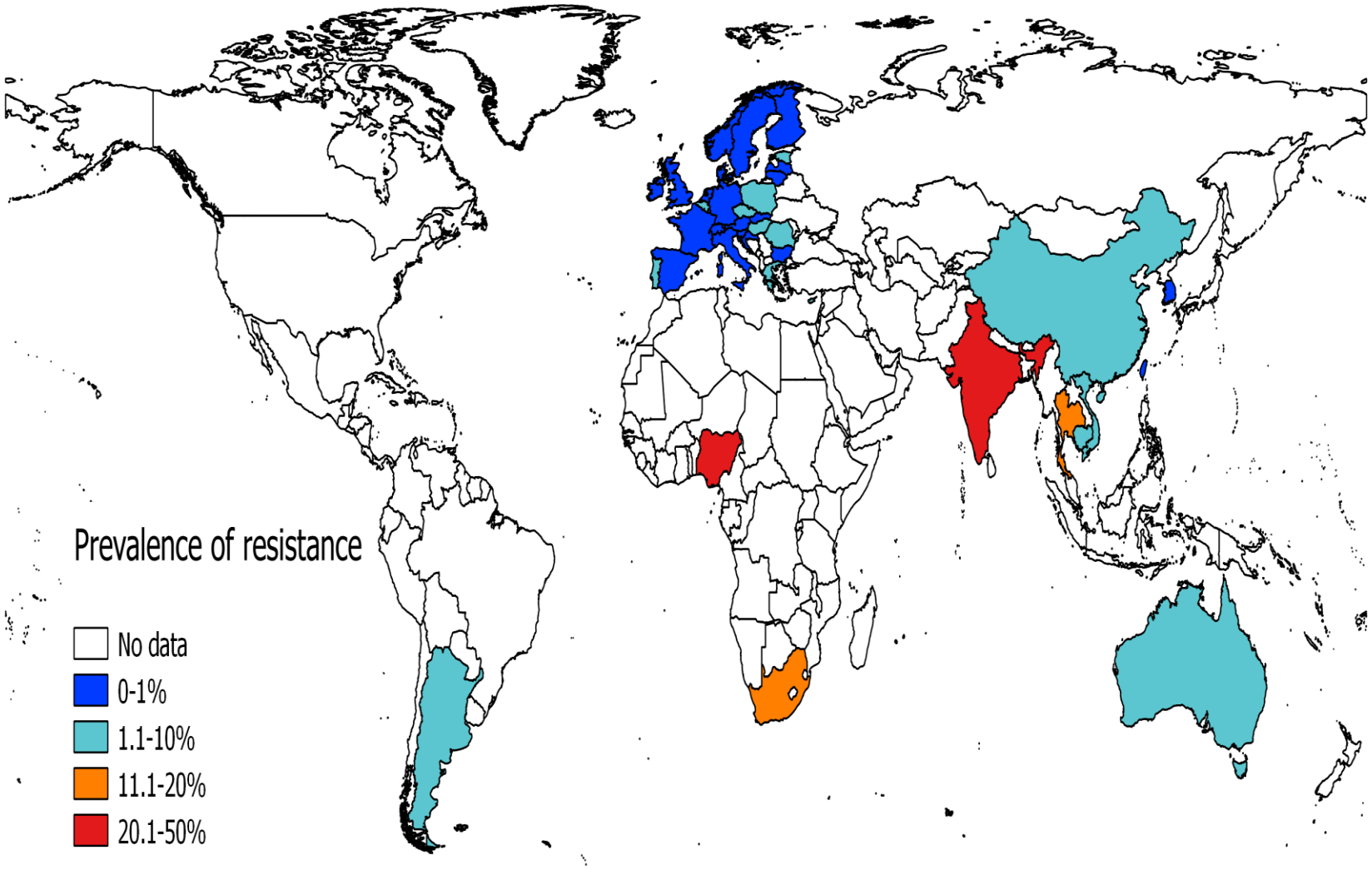


Figure 2-7. Global prevalence of colistin resistance in general-EC from healthy pigs

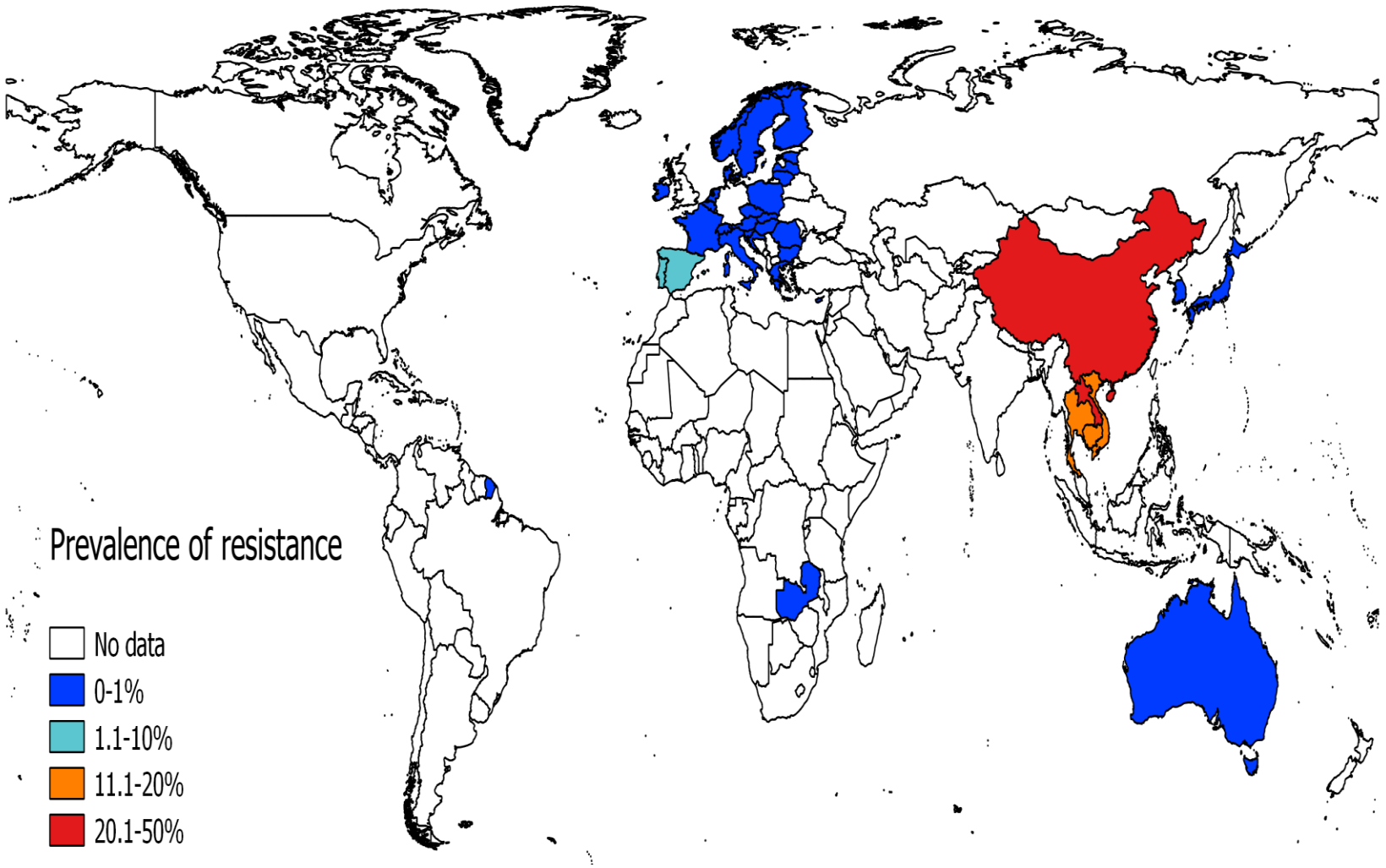


Figure 2-8. Global prevalence of colistin resistance in general-EC from diseased pigs

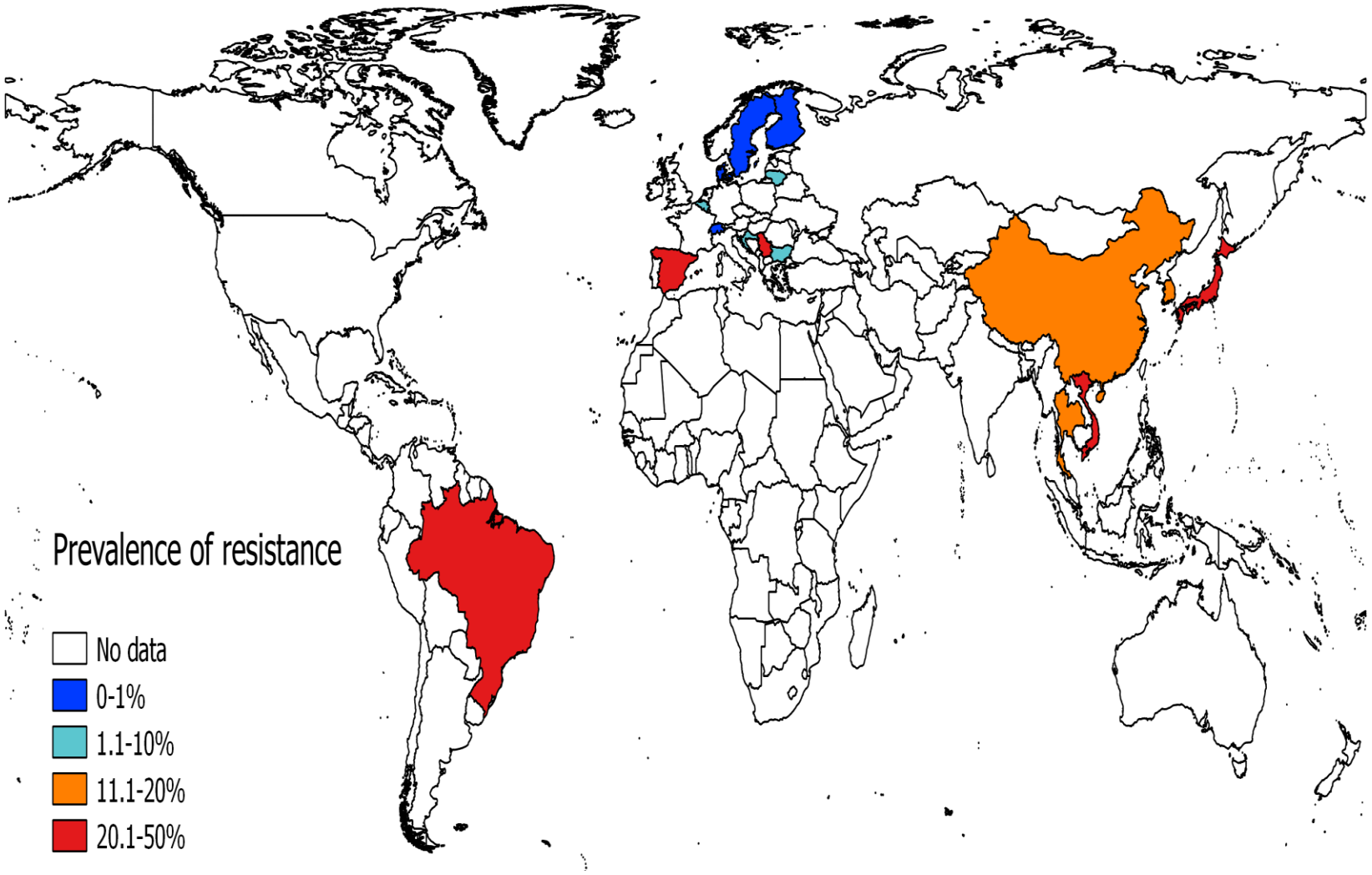


Figure 2-9. Global prevalence of FQ resistance in general-EC from healthy pigs

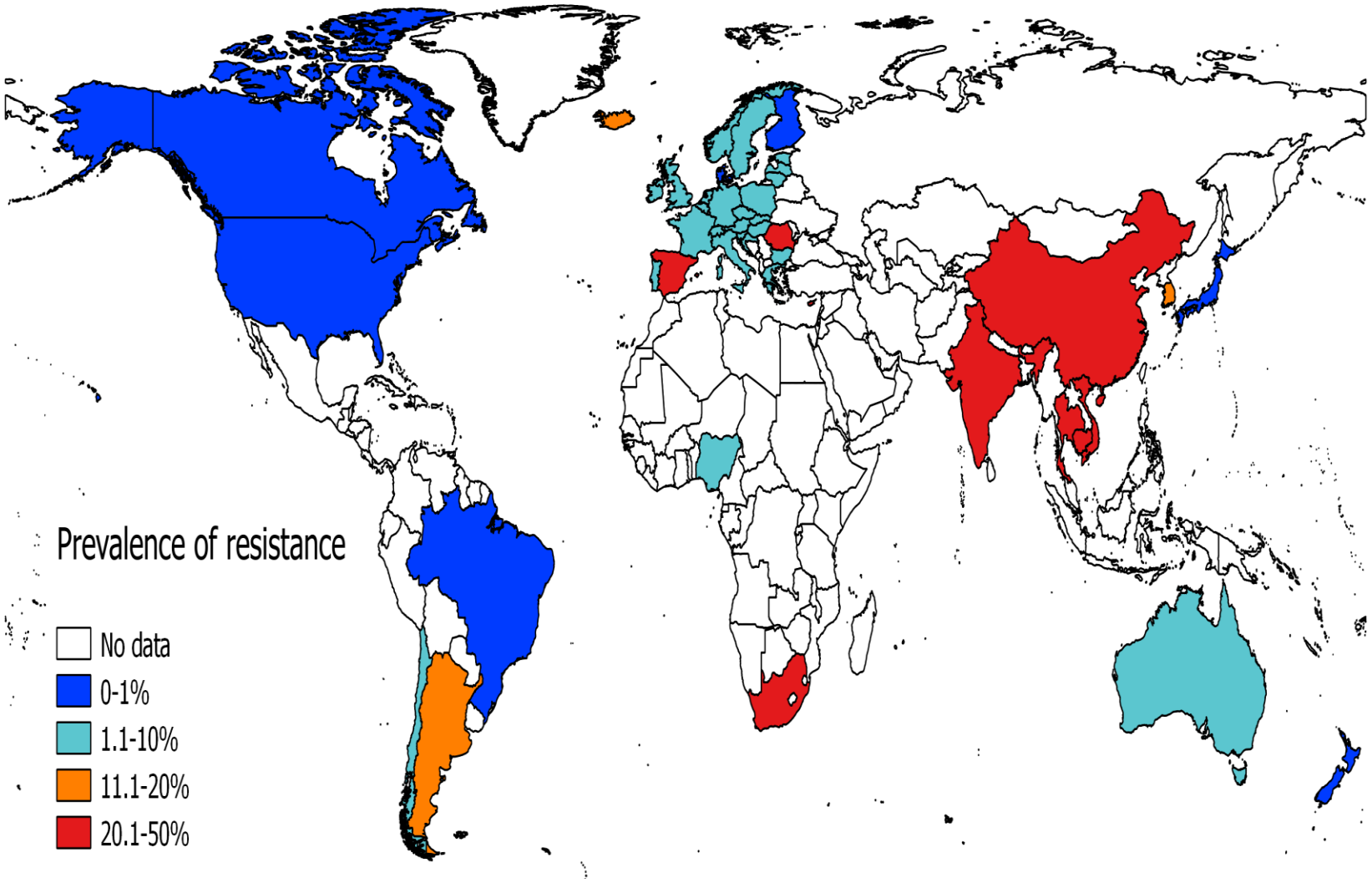
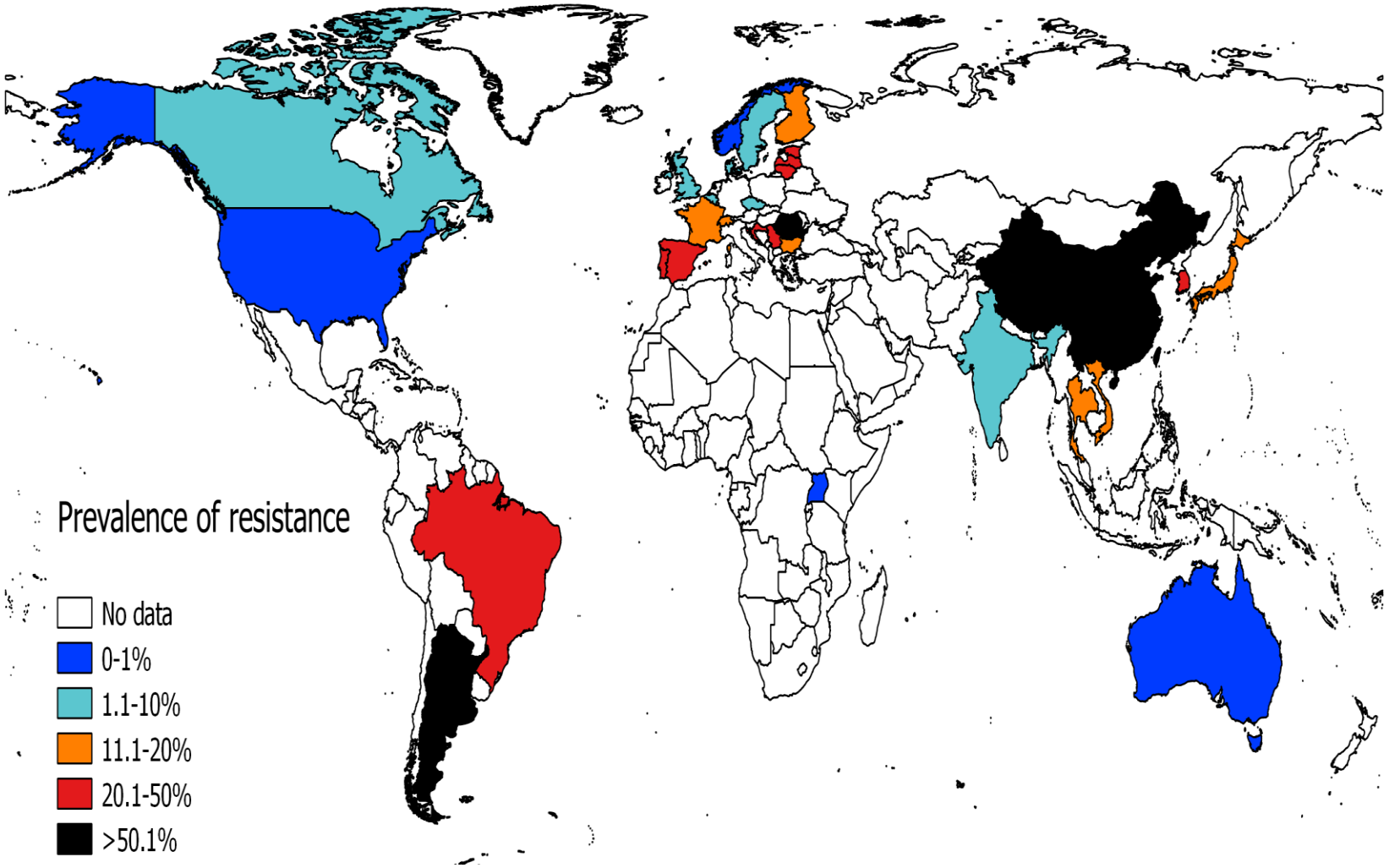


Figure 2-10. Global prevalence of FQ resistance in general-EC from diseased pigs



Supplementary material

S 2-1. Search strings used in systematic review:

Pubag- ((antibiotic or drug or antibacterial or antimicrobial or colistin or polymyxin or lactamase or beta or quinolone or fluoroquinolone or carbapenemase) and (resistance or susceptibility) and (pig or sus or porcine or swine) and (escherichia coli or e coli))

Web of science- ((TS=((antibiotic or drug or antibacterial or antimicrobial or colistin or polymyxin or lactamase or beta or quinolone or fluoroquinolone or carbapenemase) and (resistance or susceptibility) and (pig or sus or porcine or swine) and (escherichia coli or e coli)))) *AND* **LANGUAGE:** (English)

Pubmed- (("Drug Resistance"[Mesh]) or "Polymyxins"[Mesh]) AND "Swine"[Mesh] AND "Escherichia coli"[Mesh])

CABI- ((antibiotic or drug or antibacterial or antimicrobial or colistin or polymyxin or lactamase or beta or quinolone or fluoroquinolone or carbapenemase or spectrum) and (Pig or Swine or Porcine or Sus) and (resistance or susceptibility or resistant) and (escherichia coli or e coli)).af.

CHAPTER-3 Prevalence and time trend analysis of antimicrobial resistance in clinical *Escherichia coli* isolates collected from diseased pigs in USA between 2006-2016

Introduction

In the last few decades, antimicrobial resistance has emerged as one of the most important problems affecting veterinary and human medicine. Antimicrobials have been the cornerstone of modern medicine, and the emergence of widespread antimicrobial resistance (AMR) could lead to a post-antimicrobial era in which common infections and minor injuries would be deadly once again.[30] Use of antimicrobials in animal production systems has been implicated as one of the causes for increased prevalence of resistant bacteria such as *Escherichia coli,* non-typhoidal *Salmonella* etc. in both animals and humans, and it is considered a major food safety issue. [38,438]

Pork makes up 36% of the world's meat supply and USA is the world's third largest producer and consumer of pork (USDA). Antimicrobials are used in swine industry worldwide for various purposes including growth promotion, prevention and treatment of diseases.[3] In USA, nearly 37% of medically important antimicrobials sold in food animals are used in swine production.[439] Since antimicrobial use in pigs can also select for resistant zoonotic bacteria, AMR in zoonotic bacteria of swine origin are also of interest to public health.[11]

Escherichia coli is a commensal microorganism typically found in the intestinal tract of mammals and it has the potential to be pathogenic in humans and animals when it harbors certain virulence genes or possess other traits such as lower fitness costs.[440] Remarkable features such as its potential to be transferred along the food chain, ease of

isolation and its ability to easily gain and disseminate genetic elements (including AMR determinants) makes this bacterium a suitable candidate to monitor the prevalence of AMR over time.[37]

Due to the potential public health and animal health impacts of antimicrobial use in animal production systems, Food and Drug Administration (FDA) has recently taken initiatives to phase out the use of medically important antimicrobials as growth promoters in animal production effective January 2017.[6] Discontinuity on the use of growth promoters is expected to contribute to a decrease in the prevalence of antimicrobial resistant bacteria in food animals, as observed in Europe when the use of growth promoters was banned.[95] However, to assess the success of changes in such policies, information on the long-term baseline prevalence of AMR in food animals is required. Passive surveillance using veterinary diagnostic laboratory data provides a low-cost alternative to studying prevalence and trends of AMR in food animals.[441] The Veterinary Diagnostic Laboratory at the University of Minnesota (MNVDL) receives thousands of clinical samples of swine origin every year coming from all the major pork producing states in the USA, and it can be considered as a source of information regarding AMR in bacteria circulating in swine in the country.

The objective of this study was to describe the prevalence and trends of the phenotypic AMR in *E. coli* of swine origin isolated from clinical samples at the MVDL between 2006-2016. The antimicrobials studied herein are of importance to both animals (e.g. phenicols, tetracyclines etc.) and humans (e.g. analogues of fluoroquinolones, aminoglycosides, third generation cephalosporins etc. used in human medicine). The

results of this study will help in informing evidence-based antimicrobial stewardship in order to improve animal and human health.

Materials and methods

Bacteriology

E. coli isolates collected from clinical samples of swine origin submitted to the MVDL for diagnostic purposes from January 2006 to December 2016 were included in the study. These included samples varying from whole swine carcasses for necropsy, tissue or fecal samples and body fluids such as urine and semen. Multiple samples could be received from a single epidemiological unit (e.g. carcasses of piglets who have died at the same farm from diarrhea) and these samples were labelled with a single accession number. Swabs collected from samples were used to inoculate sheep blood agar and MacConkey agar plates that were incubated at 37^0 C for 18-24 hours. Colony morphology and presence/absence of hemolysis was noted, and a single colony per morphology was selected and streaked on a new MacConkey agar plate for incubation as described above. Bacterial colonies from the second MacConkey agar plate were used for antimicrobial susceptibility testing.

Antimicrobial susceptibility testing of all isolates was performed by the broth microdilution method using the Sensititre automatized dilution system (Trek Diagnostic Systems, Cleveland, OH) to determine the minimum inhibitory concentration (MIC) according to the CLSI standards (CLSI, VET01-A4). Only antimicrobials for which at least 100 isolates were tested annually over the 2006-2016 period were included in this analysis: Ampicillin (AMP, range- 0.25-16 µg/ml), Ceftiofur (XNL, range- 0.25-8 µg/ml), Chlortetracycline (CTET, range- 0.5-8 µg/ml) , Enrofloxacin (ENR, range-

0.125-2 µg/ml), Florfenicol (FFN, range- 0.25-8 µg/ml), Gentamycin (GEN, range- 1-16 µg/ml), Neomycin (NM, range- 4-32 µg/ml), Oxytetracycline (OTC, range- 0.5-8 µg/ml), Spectinomycin (SPT, range- 8-64 µg/ml), Sulphadimethoxine (SDM, range- 256->256 µg/ml) and Trimethoprim-Sulphamethoxazole (SXT, range- 2->2 µg/ml).

Selection of *E. coli* isolates for antimicrobial susceptibility testing and virulence profiling was based on bacterial morphology, discretion of diagnostic pathologist and/or request of the submitting client. Usually, one *E. coli* isolate per accession number was screened for antimicrobial susceptibility testing, particularly if all isolates from the accession had similar morphological characteristics. Beta-hemolytic *E. coli* isolates were preferentially selected because they have been associated with clinical disease in neonatal and post-weaning pigs. However, if there were multiple bacterial morphologies or if the clinical history of the pigs submitted within the same accession differed, multiple *E. coli* isolates could be selected for antimicrobial susceptibility testing.

Statistical analysis

Analyses were carried out at the isolate level. To avoid overrepresentation of the same *E. coli* strains, only one isolate per AMR profile and submitting client per month were selected for the analysis. Only isolates for which information on the date of submission (later categorized as season), age of the pig (age categories or exact age) and hemolytic nature (yes/no) (table S3-1) was available were included in the study. Isolates were classified as resistant or non-resistant based on breakpoints provided by CLSI and National Antimicrobial Resistance Monitoring Systems (NARMS) or epidemiological cut-offs provided by EUCAST (table S3-2). Isolates categorized as intermediate by breakpoints were grouped with the non-resistant group. For purpose of statistical

analyses, MIC values below/above the dilution ranges were rounded to the next serial dilution immediately before/after respectively.

Univariate analysis- Hierarchical logistic regression models with a logit link function were fitted for each antimicrobial using the binary AMR response as the outcome variable (resistant or non-resistant) and the time (in years), season, age and hemolytic nature as independent variables (package "lme4", R). Geographic state of origin of the sample were included in all models as random effects to account for the lack of independence between observations. The time associated odds ratios were estimated by exponentiating the coefficient for the time (in years) variable and the odds ratio at average prevalence levels were subsequently converted to risk ratios to estimate the percent annual change in prevalence of resistance to individual antimicrobials (package "sjstats", R).[442]

Analyses of multiple antimicrobial resistance (MAR)- MAR was studied using various statistical approaches- regression analysis, diversity index (richness of AMR combinations per year) and network analysis. For regression models, an isolate was classified to be multiple antimicrobial resistant if it was resistant to 3 or more different antimicrobial classes. Antimicrobials belonging to same class were grouped together in one class and the isolate was considered resistant to that antimicrobial class if it was resistant to at least one of the antimicrobials in that class. (CTET and OT), (GEN and NM) and (SXT and SDM) were grouped together as tetracyclines, aminoglycosides and sulphonamides, respectively. Hierarchical logistic regression model with logit link were built with MAR as binary outcome variable and time (in years) as continuous independent fixed- effects variable, age, season and hemolytic properties as categorical

independent fixed-effects variables and submitting client and geographical state as categorical, random effect variables. Rarefaction curves were built to estimate the richness or number of unique combinations of phenotypic resistances (resistotypes) per year after rarefying to control for uneven number of isolates analyzed annually (package "vegan", R).

For the network analysis approach, MIC data were analyzed using Gaussian graphical model. This is a type of undirected graphical probabilistic model which has been used to estimate partial correlations between AMRs using MIC values directly and plotting them for easy graphical inferences, as previously described using "Rnets" package in R.[12] Briefly, MIC values for each year were first converted into empirical correlation matrices using Spearman's rank correlation method. These matrices were then transformed into penalized precision matrices based on a regularization parameter λ, which reduces certain correlations to zero and hence, produces sparser but more interpretable networks. These penalized precision matrices were then plotted graphically in the form of a network wherein the vertices represent the antimicrobials and the edges represent the penalized (weighted) partial correlations. The selection of λ was done using the stability approach to regularization selection method for each year. Stability scores for various values of λ (ranging from 0.05 to 0.60 in 0.05 increments) were evaluated for each year. The smallest λ that had a stability statistic (D) <0.10 was selected to estimate penalized correlation matrices for each year.

The global network strengths of these graphical models were then compared using network comparison tests using the package "Network Comparison Test" in R. MIC values were first normalized using nonparanormal transformation and the global network

strengths were calculated as the weighted absolute sum of all edges in a regularized partial correlation network under various permutations and pairwise comparison between AMR networks from different years were compared. p-values were adjusted using the linear step-up method as previously described to decrease false discovery rates due to multiple testing (PROC MULTTEST, SAS).

Results

General description of data

There were 9697 isolates with complete information on age, state of origin of the animal from which the sample originated, date of submission, hemolytic characteristics and susceptibility results for all antimicrobials under study. On average, 881 isolates were retrieved every year (range- 434-1115 per year). The main characteristics of the isolates are provided in table S3-1. Nearly 48% of the isolates were collected from nursery pigs, followed by pre-weaning pigs (37%). Frequency of isolation was nearly equal across all four seasons. Nearly two-thirds (62%) of the isolates were beta-hemolytic. Overall, isolates were collected from accessions submitted by 242 unique clients (swine systems, veterinary clinics) spread over 35 states across USA. Nearly 39% (n=4196) isolates were collected from Minnesota. The geographic distribution of isolates collected from states other than Minnesota are presented in table S3-3.

Univariate analysis

Raw change in prevalences to antimicrobials are presented in figure 3-1 and results from hierarchical logistic regression models are presented in table 3-1. The antimicrobials evaluated here could be divided in four groups, based on the percentage of resistant isolates found. For ampicillin, chlortetracycline, oxytetracycline and sulphadimethoxine, the average percentage of resistant isolates ranged between 60-95% (figure 3-1). Except

for subtle changes, the percentage of isolates resistant to chlortetracyline and oxytetracycline remained nearly constant. However, the percentage of isolates resistant to AMP increased from ~67% between 2006-13 to ~75% by 2016. In contrast, the percentage of resistant isolates to SDM decreased from 81% in 2006 to 65% in 2016 (annual decrease in prevalence-3%, $p<0.01$).

The prevalence of SPT resistance averaged at 40.5% and this prevalence decreased consistently from 49.4% in 2006 to 37.1% in 2016 (annual decrease in prevalence -3%, $p<0.01$). The percentage of isolates resistant to florfenicol, gentamicin, trimethoprim-sulphamethoxazole and ceftiofur ranged between 18-40% during the study period (figure 3-1), with values fluctuating over the years, except for florfenicol resistance which decreased steadily from 31% in 2007 to 19.5% in 20-15 (annual decrease in prevalence -3%, $p<0.01$). Finally, in the case of enrofloxacin, a steady increase in the percentage of resistant isolates was observed between 2006 (~1%) and 2016 (21%) (annual increase in prevalence-39%, $p<0.01$).

Analyses of MAR

Up to 264 (2.7%) and 1,136 (11.7%) of the isolates were either fully susceptible to all antimicrobials or resistant to only one antimicrobial class, respectively. Overall, there were 122 unique resistotypes. The top ten resistotypes, which accounted for up to 57% of the isolates (n=5,531) are shown in table 3-2. The mean and median number of antimicrobial classes that the isolates were resistant to was 3.81 and 4, and these numbers remained nearly constant across the years. However, based on rarefaction curve, the number of unique resistotypes increased consistently from 43.8 in 2006 to 69.4 in 2016 (mean= 56) for the same number of isolates per year (n=434) (figure 3-2).

There were 6,956 (72%) isolates which were resistant to ≥ 3 antimicrobial classes. Based on a hierarchical logistic regression model, the percentage of isolates resistant to 3 or more antimicrobial classes decreased by 1.71% annually (95% CI- 0.36 to 3.05%) (p-value=0.01).

The optimum λ-value based on the stability statistic (first D value < 0.10) varied across years and was 0.30, 0.35 and 0.40 for 6 years, 5 years and 1 year, respectively. The number of edges per year in the networks based on different values of λ are presented in figure 3-3 and an increase in the number of edges over the years can be seen in AMR networks regardless of λ-value. Networks built with λ-value=0.3 are presented in figure 3-4 and these networks were used for comparative purposes below.

At the value of λ=0.3, 22 unique edges were found out of possible 45 edges (48.9%) across all years. The mean density of the networks was 27.3%, ranging from 20% in 2007 and 2009 to 35.6% in 2015. The average number of closed triplets (3 AMR nodes connected to each other) was 2.4 per network for 2006- 2010 and increased to an average of 6.83 per network for 2011-2016. Furthermore, there were sub-graphs of 4 AMR nodes interconnected to each other in years 2011, 2014 and 2015. For 2016, 5 AMR nodes (GEN-NM-SDM-SPT-SXT) were interconnected to each other and there were 11 closed triplets in that year. The most common closed triplets were GEN-SPT-SXT (years 2006, 2010-2016), GEN-NM-SXT (years 2008, 2011, 2012, 2014-2016), SDM-SPT-SXT (years 2011-2016) and AMP-FFN-XNL (years 2006, 2009-2012). Comparison of networks revealed that there were no statistically significant differences in network strengths between 2006-2010 (figure 3-4). Significant differences ($p<0.05$) in network strengths were found for ten pairs of years: 2006-2011, 2007-2011, 2008-2011,

2008-2012, 2007-2013, 2008-2013, 2007-2014, 2008-2014, 2007-2016 and 2008-2016 (figure 3-4).

Discussion

The objectives of this study were to describe the prevalence and evolution of AMR in *E. coli* isolates recovered from swine clinical samples submitted to the MVDL between 2006-2016. Prevalence of resistance to certain antimicrobials such as tetracyclines, ampicillin, sulphamethoxazole was found to be high throughout the period of study (>50%). There was either a modest decrease or increase in prevalence of resistance to all antimicrobials, except for prevalence of enrofloxacin resistance which increased drastically during the study period. Similarly, the proportion of isolates resistant to more than 3 antimicrobial classes decreased modestly but the networks of MARs became denser and resistotype richness increased over time.

The prevalences of ampicillin and gentamicin resistances in our study were similar to those found in swine clinical *E. coli* isolates from Ontario, Canada and Australia but prevalence of ceftiofur resistance was higher in our study as compared to these studies (Canada- 10-20%, Australia- 0%).[130,443] The prevalence of neomycin resistance in this study was higher than in the United Kingdom (18%) but lower than in Australia (42%).[130,308] The prevalence of resistance to other antimicrobials (sulphonamides, tetracyclines) was similarly found to be high (>50%) in swine clinical *E. coli* isolates from Australia, Canada, France, Italy and the United Kingdom.[130,198,308,443,444] Also, prevalences of florfenicol and spectinomycin resistance in current study were similar to those estimated in Denmark and Canada.[190,443] Considering that sulphonamides, tetracyclines and penicillins are estimated to be among the top classes of antimicrobials used in food animal production medicine in USA, Canada and several European

countries, the high prevalence of resistant isolates found here might be potentially correlated with their consumption in food animals.[119,161,439]

Similar to our study, the prevalence of ceftiofur, potentiated sulphonamides, gentamicin and tetracycline resistance did not change significantly or changed only to a modest extent in clinical swine *E. coli* isolates from Canada and France over a long period of time, suggesting that the resistance to certain key antimicrobials has remained stable worldwide including USA.[198,443] The prevalence of AMRs also seldom remained constant and fluctuated greatly over short time in these studies. Using a shorter timeframe for AMR trend analysis could lead to raising of false alarms. Currently, EFSA comducts trend analysis on AMR data collected for 5 years or more.[445]

Fluoroquinolones such as enrofloxacin are classified as critically important for human health[30] and are also effective for treating several bacterial infections in animals.[446] The percentage of resistance to enrofloxacin increased among the *E. coli* evaluated here rapidly from 1% in 2006 to 21% in 2016 (39% increase annually). These results are similar to that what has been described in swine clinical *E. coli* isolates from several European countries, but considerably higher than that from Australia and Canada, where nearly 0% of isolates are enrofloxacin resistance (Chapter-2). A drastic increase in enrofloxacin resistance was also reported in *Salmonella spp.* isolates in USA[447] and *E. coli* isolates in Italy.[444] In USA, enrofloxacin was labelled for use as a treatment of swine respiratory disease complex in 2008 and enteritis in 2012[447], thus suggesting that the increase in resistance may be linked to its use in swine medicine.

The problem of AMR is further compounded by the phenomena of co-resistance which enables an isolate to be resistant against multiple antimicrobials by acquiring

multiple mobile genetic elements or chromosomal mutations (Canton and Ruiz-Garbajosa, 2011).[448] Similar to our study, patterns of co-resistance to penicillin-sulphonamide-other antimicrobials and tetracycline-sulphonamide-other antimicrobials were reported to be common in swine clinical *E. coli* isolates from Sweden and Denmark, respectively.[190,303] Additionally, AMR networks revealed potential correlations between resistances to different antimicrobials such as ceftiofur-florfenicol, enrofloxacin-trimethoprim-sulphamethoxazole etc. These co-resistances can have practical implications on control of AMR as use of one antimicrobial class can select for an unrelated antimicrobial class, which can have consequences on policies relying on restriction of antimicrobial usage.[448] Sundqvist et al. (2010) cited co-resistance with multiple antimicrobials as one of the reasons behind persistence of trimethoprim-sulphamethoxazole resistance in *E. coli* despite drastic reduction in its use in human populations in the UK.[449] Several field trials have demonstrated the correlation between resistance to an antimicrobial and use of an unrelated antimicrobial in animal production systems.[11,450]

There was a slight annual decrease in proportion of isolates resistant to more than 3 antimicrobial classes but there was an increase in resistotype richness over time implying an increase in the number of co-resistance patterns over the years. An increase in density of AMR networks further proves that the correlations between resistances to muktiple antimicrobials have increased statistically. The differences in results using logistic regression and network analysis can be explained by loss of information due to dichotomization of AMR results when evaluating the evolution in AMR.[451] Dichotomizing AMR data to classify isolates as resistant to multiple antimicrobials has

been a common method to characterize and study changes in MAR.[452] Considering that multivariate networks approach uses the complete spectrum of MIC values and provides information about biological relationships between different AMRs, we recommend using this approach in conjunction with the traditional methods (logistic regression). However, biases can be induced in the interpretation of the network analysis method used in this study as the results are highly sensitive to value of λ used. As a result, it is recommended that data on the density of networks based on different values (figure 3-3) should be provided along with the network graphs.

There are a few key points that must be considered before making inferences based on this study. First, the MVDL represents a passive surveillance system and hence, there are inherent biases in the samples received here, such as higher number of isolates from Minnesota, preferential selection towards hemolytic *E. coli* strains, etc. that can potentially affect the generalizability of the results. Secondly, the isolates are typically collected from diseased animals which might have been treated with antimicrobials prior to being sampled. Indeed, the prevalence of AMR in our study is considerably higher than that in *E. coli* isolates collected from healthy pigs at slaughter between 2013-16.[453] As such, this study should not be interpreted as an analysis of clinical efficacy of these antimicrobials in the field. Lastly, there is a need to correlate these prevalences and trends in AMRs with actual antimicrobial usage data. Availability of antimicrobial usage data in swine production medicine and field trials to study effects of using antimicrobials on co- and cross-selection of AMR in USA can help to clarify the possible role of antimicrobial use in the selection of resistant bacteria in swine.

Despite these important limitations, we believe this study provides valuable information in several ways. Despite the difference in levels of prevalences between our data and *E. coli* isolates collected from pigs at slaughter during active surveillance (NARMS), the rank order of prevalence of different AMRs is similar in both datasets e.g. penicillins, tetracyclines and sulphonamide are also the top three resistances in NARMS data.[453] It has been postulated that analyzing trends in AMR using passive surveillance is beneficial and more powerful in detecting early, emerging but rare AMRs as compared to active surveillance.[9] Indeed, the emergence and rapid increase in fluoroquinolone (enrofloxacin) resistance in this study preceded the emergence of fluoroquinolone resistance in *Salmonella* spp. isolates collected from healthy swine and retail pork by NARMS.[454] Hence, passive surveillance of AMR can serve as an important ancillary tool to the active surveillance programs.

Conclusion

In conclusion, our study elucidated changes in patterns of AMR in swine clinical *E. coli* isolates collected over more than a decade. For the antimicrobials included in this study, prevalence of AMR remained constant or changed modestly, except for enrofloxacin resistance. However, emergence in enrofloxacin resistance, persistence of high prevalences of AMR to ampicillin, sulphadimethoxine and tetracyclines and increase in resistotype richness and density of AMR networks over the years could be a cause of concern for controlling AMR in swine. These results can help in guiding surveillance of AMR in swine production and conservation of antimicrobials' efficacy by informing judicious use of antimicrobials. However, the lack of associated antimicrobial usage data hinders the interpretation of these results. Future studies should aim at associating

antimicrobial usage data with results of AMR prevalence and field trials are needed to study the extent of co-resistance in selection of AMR in actual production settings.

Funding

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Tables

Table 3-1. Changes in prevalence of AMR based on univariate hierarchical logistic regression models

Antimicrobial	Overall percentage of resistance	Range of annual percentage resistance	Risk ratio* (95% CI)
Ampicillin	68.1	63.3-75.4	**1.01 (1.01-1.02)**
Ceftiofur	34.1	29.8-39.1	0.99 (0.98-1.0)
Enrofloxacin	8.50	0.2-21.5	**1.39 (1.35-1.44)**
Florfenicol	24.1	19.5-31.0	**0.97 (0.96-0.98)**
Gentamicin	23.9	17.5-29.2	**1.02 (1.01-1.04)**
Neomycin	33.8	25.6-40.2	**0.98 (0.97-0.99)**
Spectinomycin	40.5	49.4-35.6	**0.97 (0.96-0.97)**
Sulphadimethoxine	71.6	65-83.3	**0.97 (0.97-0.98)**
Oxytetracycline	92.8	90.6-95.8	**1.0 (0.99-1.0)**
Trimethoprim-Sulphamethoxazole	22.0	18.2-28.6	1.01 (1.0-1.02)
Chlortetracycline	82.0	78.1-89.0	1.0 (0.99-1.0)

Numbers in bold were significant at $p<0.05$

*- Risk ratio was obtained by converting odds ratio associated with time (in years) at average prevalence of individual AMR from hierarchical logistic regression models with

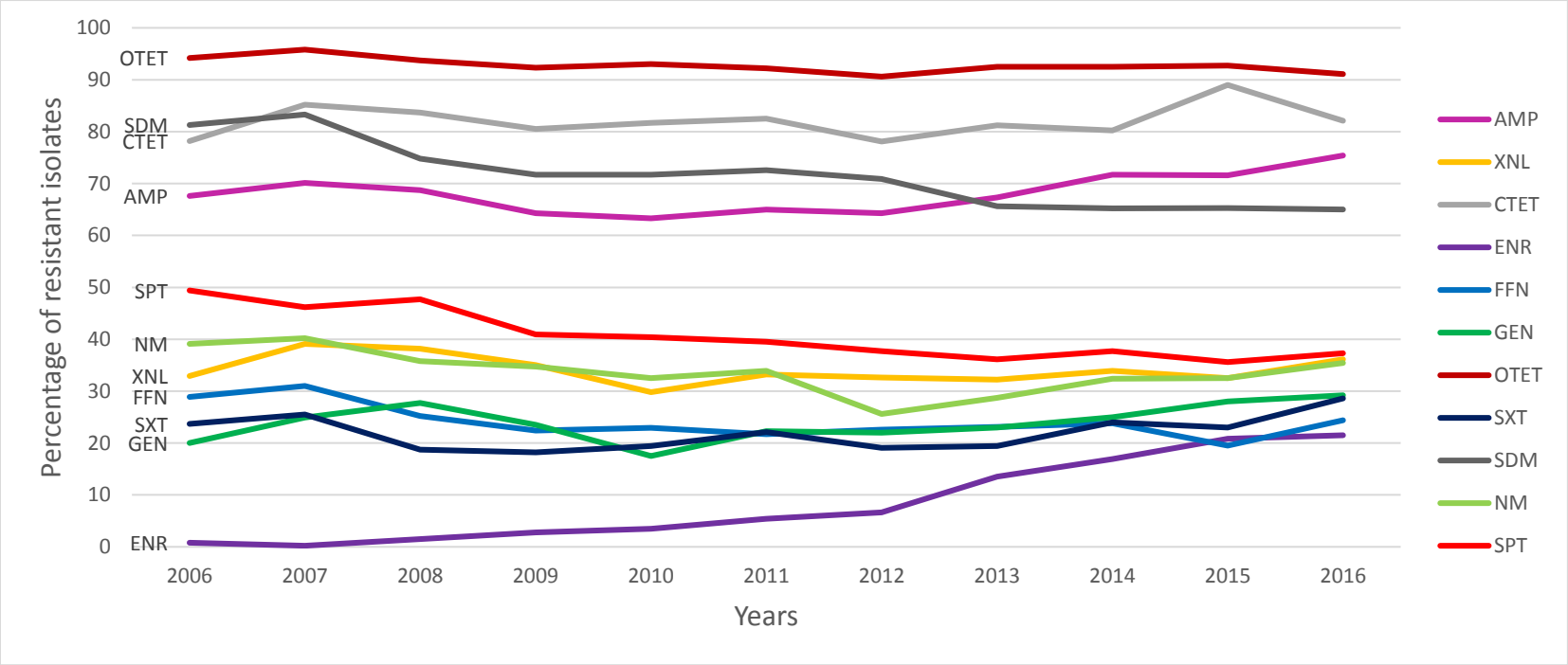
season, hemolytic characteristics and age as other fixed effects and geographic state of collection as random effect.

Table 3-2. Most common resistotypes

Resistotype	Number of isolates
Tetracycline only	964
Penicillin-Aminocyclitol-Tetracycline-Aminoglycoside-Sulphonamide	834
Penicillin-Cephalosporin-Phenicol-Aminocyclitol-Tetracycline-Aminoglycoside-Sulphonamide	692
Penicillin-Cephalosporin-Phenicol-Tetracycline-Sulphonamide	641
Tetracycline-Sulphonamide	497
Penicillin-Tetracycline	485
Penicillin-Tetracycline-Sulphonamide	437
Penicillin-Cephalosporin-Aminocyclitol-Tetracycline-Aminoglycoside-Sulphonamide	333
Penicillin-Aminocyclitol-Tetracycline-Sulphonamide	329
Aminocyclitol-Tetracycline-Sulphonamide	319

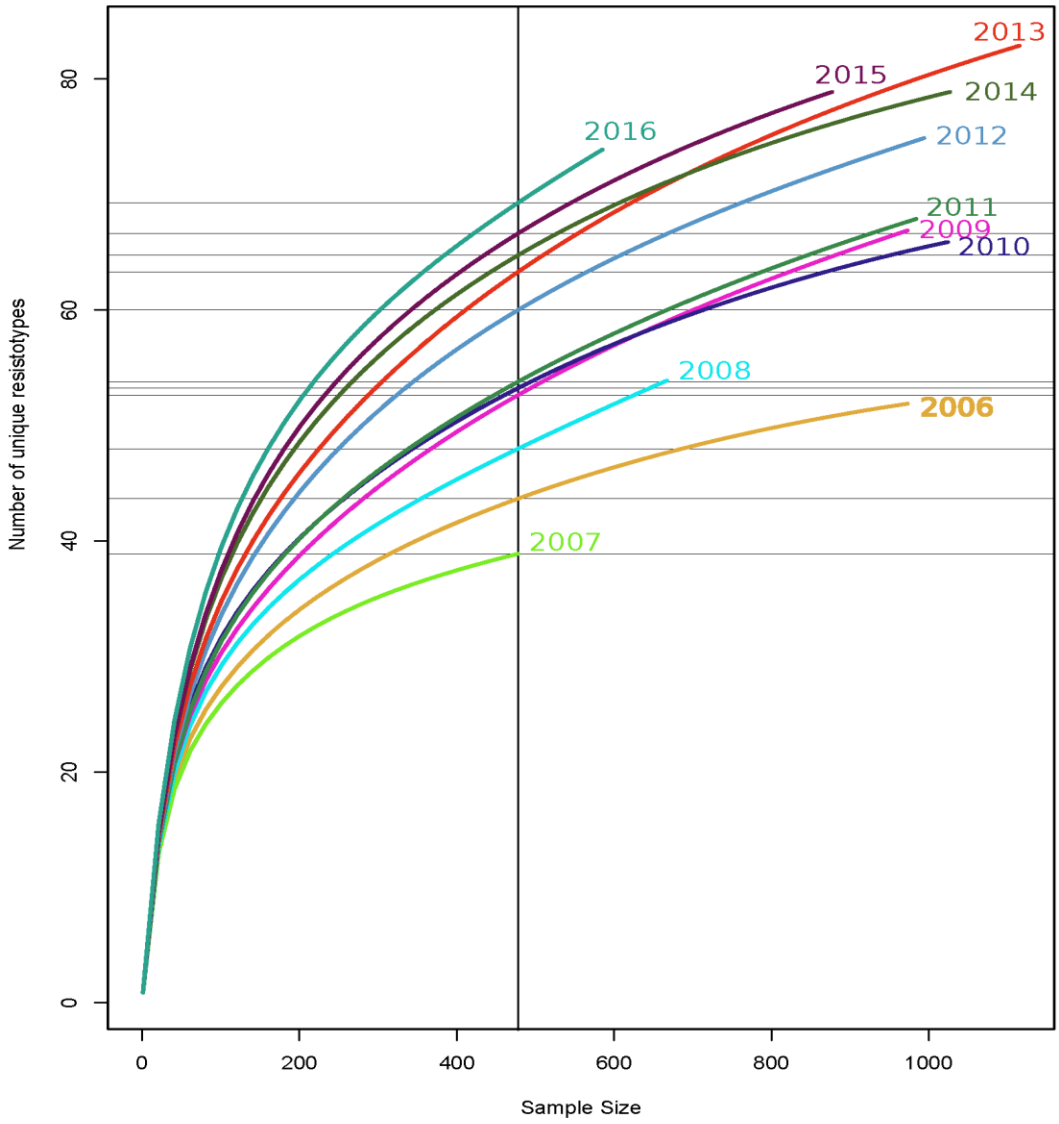
Figures

Figure 3-1. Changes in prevalence of AMRs over years



Vertical axis represents percentage of resistant isolates, horizontal axis represents the year of isolation, legend on the right represents antimicrobials tested- AMP (ampicillin), XNL (ceftiofur), CTET (chlortetracycline), ENR (enrofloxacin), FFN (florfenicol), GEN (gentamicin), OTET (oxytetracycline), SXCT (trimethoprim-sulphamethoxazole), SDM (sulphadimethoxine), NM (neomycin) and SPT (spectinomycin).

Figure 3-2. Rarefaction curve displaying number of unique phenotypes per year



Vertical axis represents the number of unique resistotypes estimated by rarefaction. Horizontal axis represents the number of isolates for which this estimation was rarefied. The vertical line in the middle starts at sample size 434 on horizontal axis, which is the least number of isolates collected for a year (2007). This line represents the number of resistotypes for different years if all the years has same sample size (n=434 isolates).

Figure 3-3. Changes in number of edges of multivariate networks of AMRs based on different values of lambda

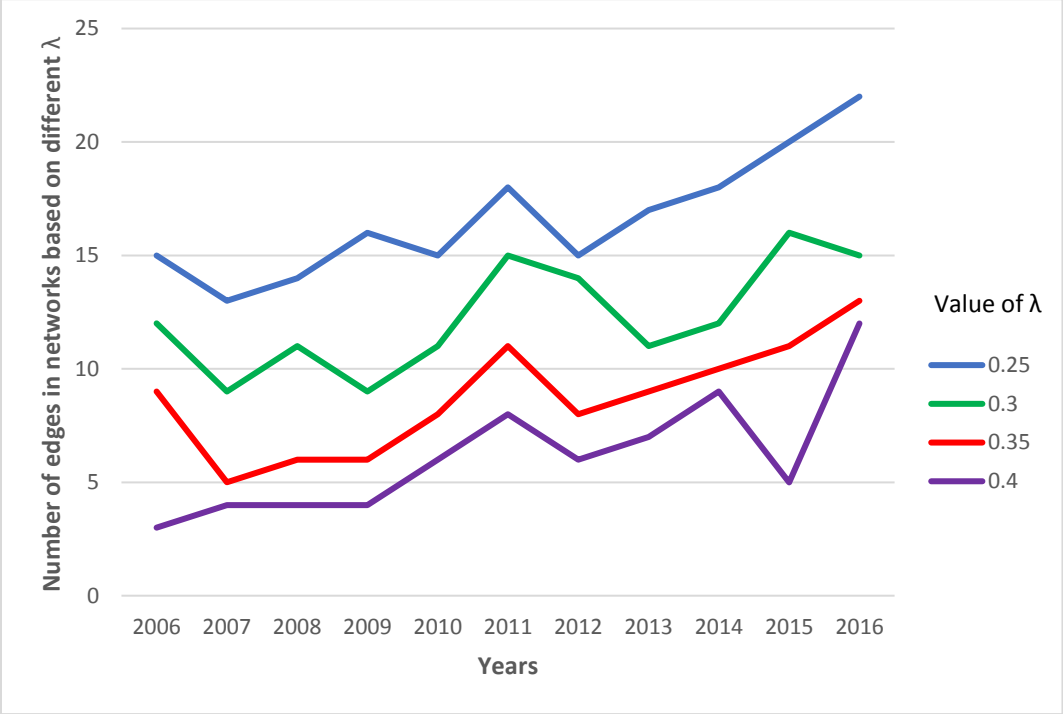
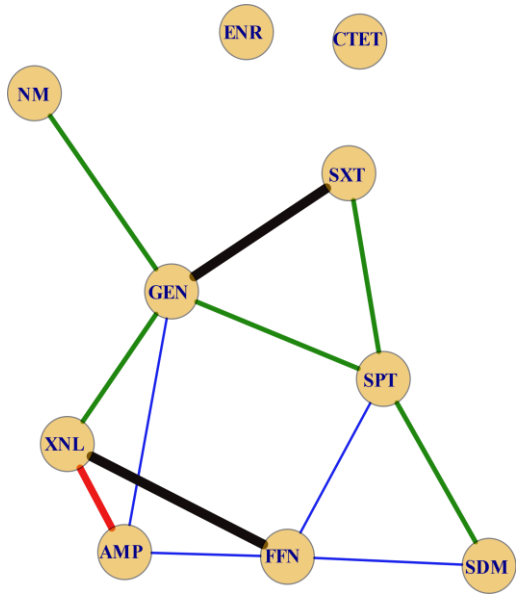
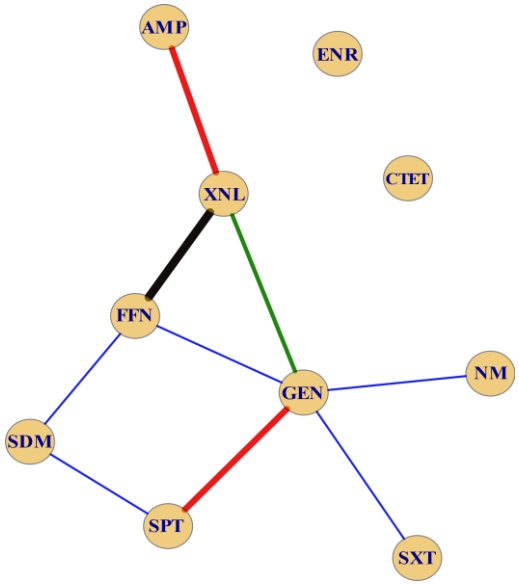


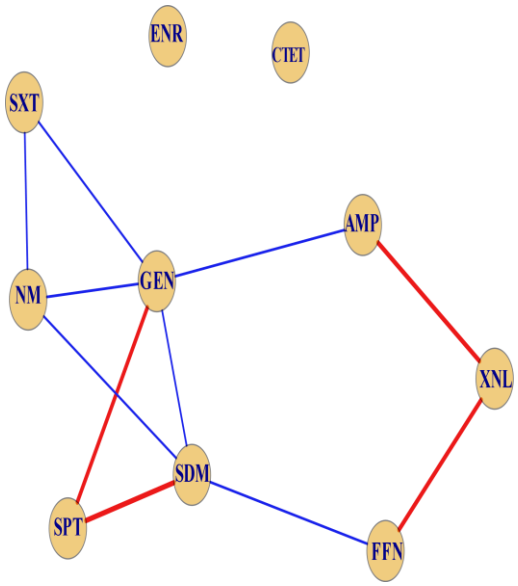
Figure 3-4. Multivariate networks of AMR



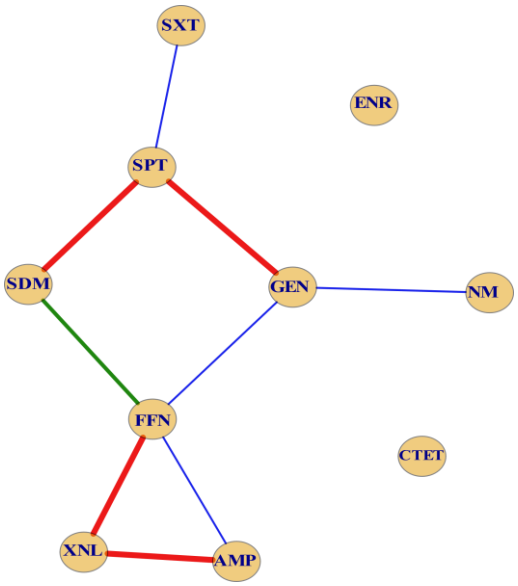
2006[ac]



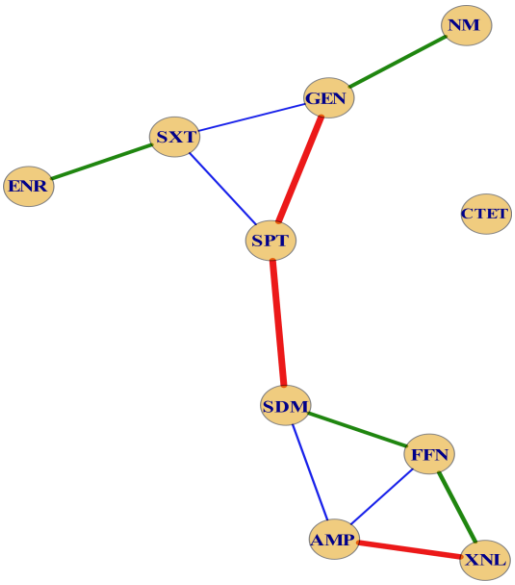
2007[a]



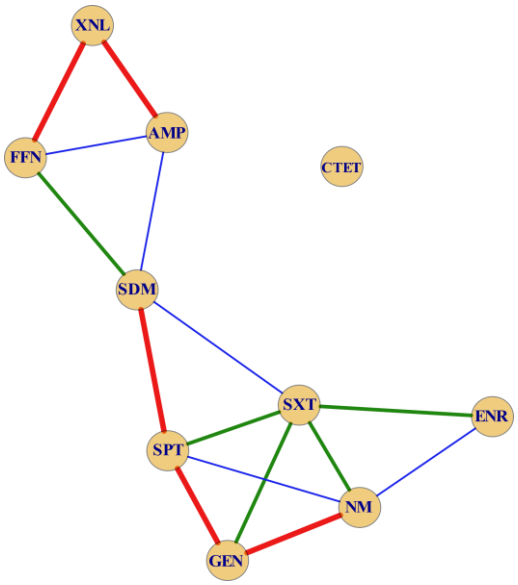
2008[a]



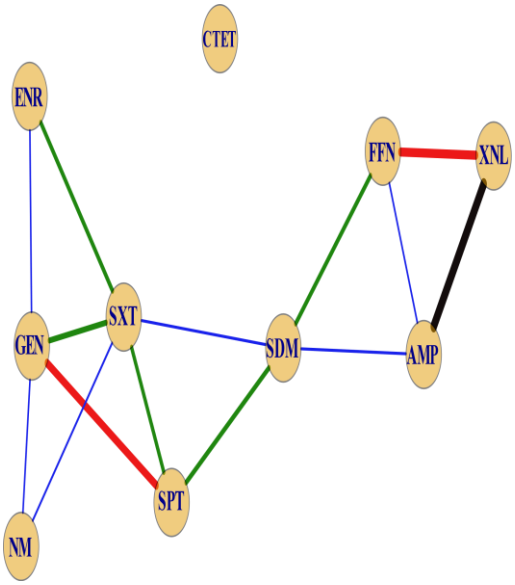
2009[ab]



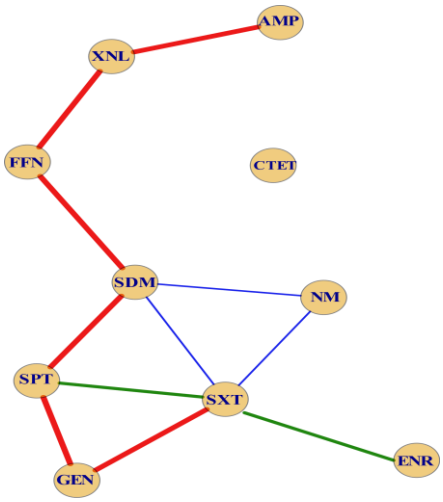
2010[ab]



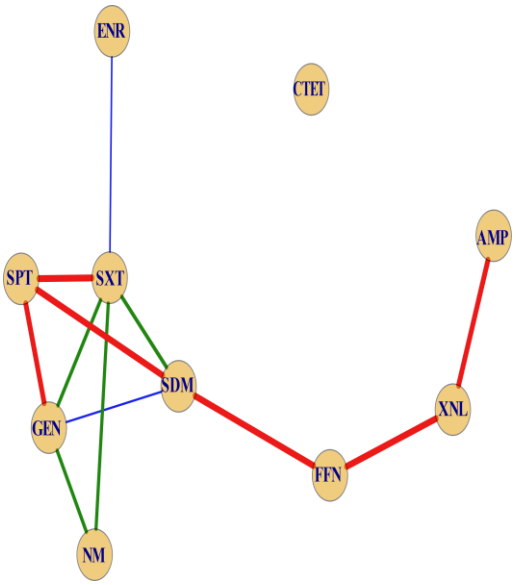
2011[b]



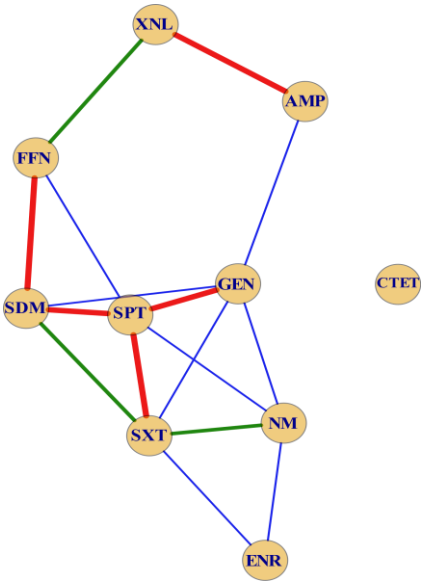
2012[bc]



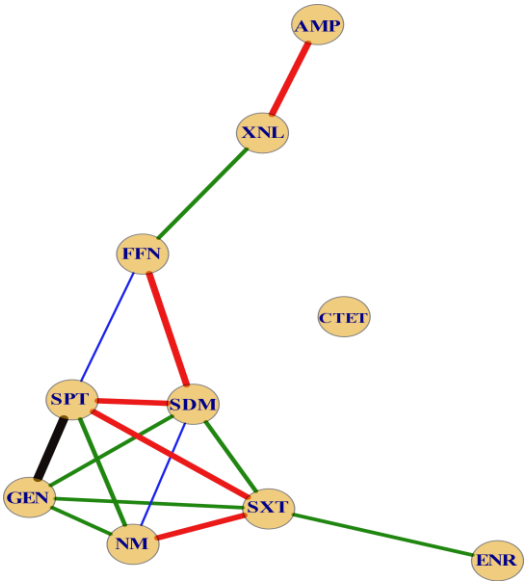
2013[bc]



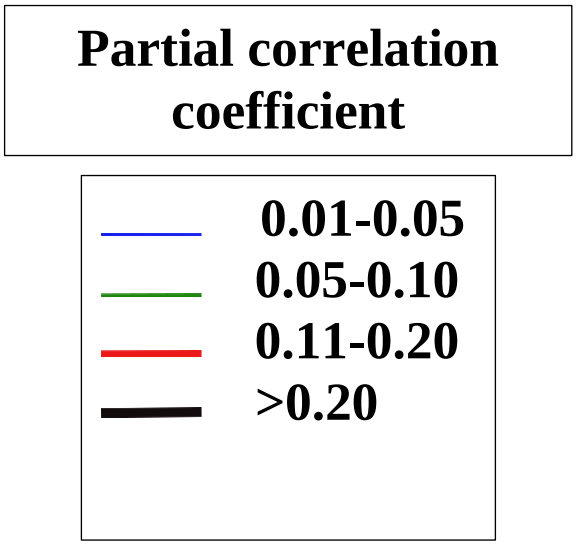
2014[bc]



2015[abc]



2016[bc]



Years with at least one of the superscript alphabets common are statistically not different (p>0.05)

Supplementary materials

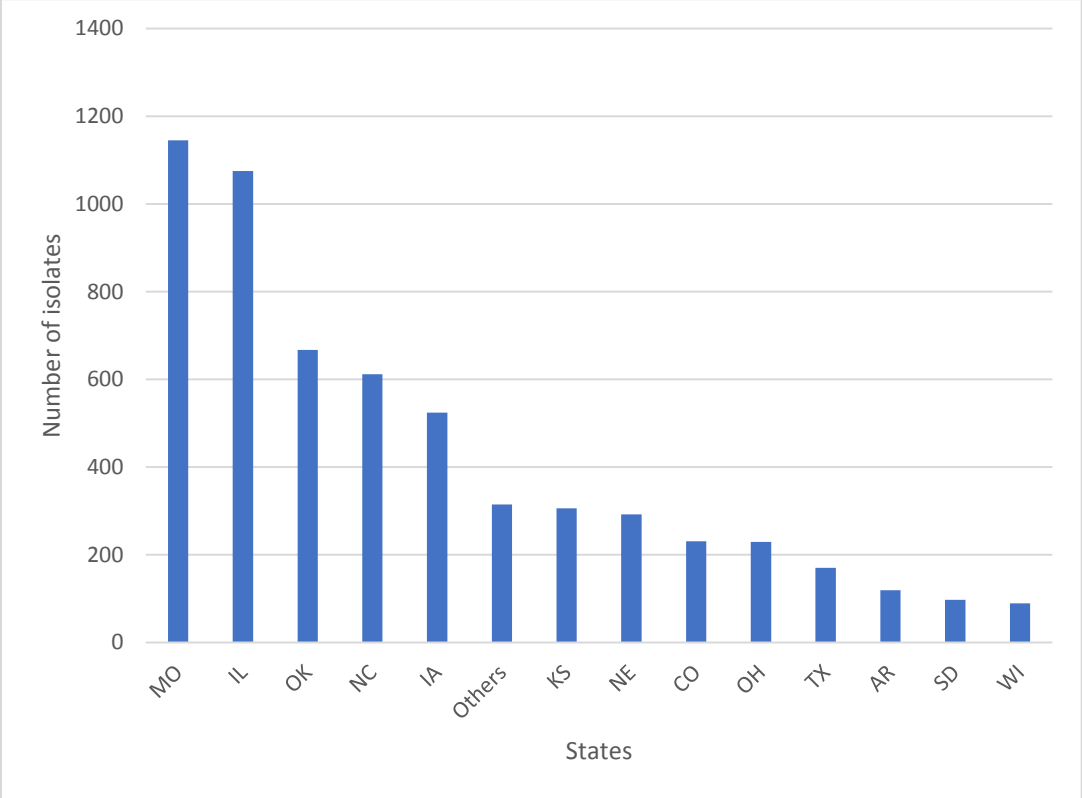
Table S3-1. Brief characteristics of isolates studied

Characteristic	Basis of classification	Number of isolates over 11 years	Percentage of total isolates (Range of isolates belonging to this category over years)
a) Age			
Fetus	Aborted material	180	1.96 (0.56-3.08)
Pre-weaning	0-21 days old	3599	37.0 (33.3-41.9)
Nursey	22-77 days old	4630	49.9 (42.2-52.9)
Growing-Finishing	78- 197 days old	1166	11.8 (8.52-15.6)
Adult	<197 days old	122	1.36 (0.78-1.95)
b) Season			
Winter	January-March	2529	26.2 (23.4-30.3)
Spring	April-June	2845	28.5 (25.1-39.7)
Summer	July-September	2168	22.0 (19.4-28.2)
Fall	October-December	2155	23.3 (8.72-27.0)
c) Hemolysis			
Beta-hemolytic		6042	62.6 (56.2-67.2)
Hemolytic		3655	37.4 (32.8-43.8)

Table S3-2. Breakpoints used for classifying isolates

Antimicrobial	Breakpoints		Source
Ampicillin	≤ 16	≥ 32	CLSI Vet08 4th edition, humans; NARMS
Ceftiofur	≤ 4	≥ 8	CLSI Vet08 4th edition, swine (respiratory infections); NARMS
Enrofloxacin	≤ 1	≥ 2	CLSI Vet08 4th edition, poultry
Florfenicol	≤ 8	≥ 16	CLSI Vet08 4th edition, swine (respiratory infections)
Gentamicin	≤ 4	≥ 8	CLSI Vet08 4th edition, humans; NARMS
Neomycin	≤ 8	≥ 16	Epidemiological cut-offs (EUCAST)
Spectinomycin	≤ 32	>64	Epidemiological cut-offs (EUCAST)
Sulphadimethoxine	≤ 256	> 256	Hong et al. (2016); NARMS
Oxytetracycline	≤ 8	≥ 16	CLSI Vet08 4th edition, humans; NARMS
Trimethoprim-Sulphamethoxazole	≤ 2	> 2	Hong et al. (2016); NARMS
Chlortetracycline	≤ 8	≥ 16	CLSI Vet08 4th edition, humans; NARMS

Figure S3-1. Geographic distribution of isolates



Horizontal axis represents the geographical location of *E. coli* isolation in USA (MO-Missouri, IL-Illinois, OK-Oklahoma, NC-North Carolina, Others-Other states, KS-Kansas, NE-Nebraska, CO-Colorado, OH-Ohio, TX-Texas, AR-Arkansas, SD-South Dakota, WI-Wisconsin). Vertical axis represents the overall number of *E. coli* isolates collected from these states between years 2006-2016.

CHAPTER-4 Prevalence and time trend analysis of antimicrobial resistance in swine respiratory bacterial pathogens collected in USA between 2006-2016

Introduction

Respiratory diseases are frequently associated with substantial financial losses in swine production systems worldwide due to high morbidity, decreased weight gain, increased culling rates, and additional medicine and labor costs.[455] Etiology of respiratory diseases is often complex, with a group of bacteria (such as *Actinobacillus pleuropneumoniae*) and viruses acting as primary pathogens with potential to cause the disease alone and other microorganisms (such as *Pasteurella multocida, Haemophilus parasuis* and *Streptococcus suis*) acting as secondary pathogens that further aggravate the disease.[456] *A. suis* and *H. parasuis* are members of the *Pasteurellaceae* family and are associated with systemic diseases including polyserositis, pleuritis, meningitis, arthritis, and respiratory diseases such as acute pneumonia.[457] *P. multocida* are commensal bacteria that can act as opportunistic secondary pathogens during respiratory diseases caused by other agents such as *Mycoplasma hyopneumoniae*.[458] *S. suis* is a Gram-positive bacteria that primarily causes septicemia, but that can also contribute to the swine respiratory disease complex (SRDC) as a secondary pathogen.[459]

Management practices, vaccination, and antimicrobial therapy are the main methods available to prevent, treat, and/or control infection caused by those pathogens. These bacteria are commensals that colonize pigs early in their life, so that subclinically infected pigs can act as sources of infection for other susceptible pigs.[460–463] Hence, management strategies aimed at preventing their circulation in a herd can be difficult to

execute in practice due to their widespread presence. The effect of vaccination in preventing and controlling infections due to these bacterial pathogens has so far proven inconsistent because of the wide serotype diversity of the bacteria involved, poor cross-protection, low protective efficacy, and interference with maternal antibodies.[464–467]

Because of the challenges in preventing infection with these ubiquitous pathogens through management measures and the inefficacy of vaccination, use of antimicrobials plays a key role in the control of SRDC. A number of antimicrobial compounds from different classes have been licensed for SRDC treatment, including penicillins (ampicillin, penicillin), cephalosporin (ceftiofur), tetracyclines (oxytetracycline, chlortetracycline), macrolides (tulathromycin), and pleuromutilins (tiamulin). Use of antimicrobials has been linked to the eventual development of resistance in bacteria,[468] and therefore there is a need for its judicious use to preserve their efficacy.[116] Surveillance of antimicrobial resistance (AMR) plays an important role in ensuring the long-term efficacy of antimicrobials but, unlike AMR in *Escherichia coli* and *Salmonella enterica*, which is routinely monitored as part of the National Antimicrobial Resistance Monitoring Systems (NARMS), little information is available regarding AMR trends in SRDC pathogens in USA.[469]

The surveillance of AMR in swine bacterial pathogens is further compounded because of the lack of clinical and epidemiological cut-offs that allow the classification of isolates as susceptible/resistant or wild type/non-wild type for several bacteria-antimicrobial combinations.[470] To circumvent this problem, clinical breakpoints of related bacterial species or newly set cut-offs are often used.[116] However, harmonization and standardization of AMR data across studies is difficult to achieve.

The objectives of this study were to estimate the prevalence of, and changes over time in AMR to antimicrobials in swine bacterial pathogens (*A. suis*, *H. parasuis*, *P. multocida* and *S. suis*) in the U.S. using antimicrobial susceptibility testing results from the University of Minnesota-Veterinary Diagnostic Laboratory (UMN-VDL) through a decade (2006-2016). The antimicrobials tested for susceptibility are commonly used in swine medicine in the U.S. Minnesota is the third largest pork producing state and the UMN-VDL routinely receives diagnostic submissions from all the major pork producing states in the U.S., resulting in an alternative source of data for analyzing AMR in swine pathogens in the absence of formal systems to collect such information in the country. Results here will help to elucidate the trend of AMR in SDRC in the U.S, which, ultimately, will help to inform decisions related with the prevention and control of one of the syndromes that results in the largest financial impact for the swine industry of the country.

Materials and methods

Bacteriology

Data on the antimicrobial susceptibility testing performed on bacteria associated with the SRDC recovered from swine samples submitted for routine diagnostic purposes to the UMN-VDL between January 1, 2006 to December 31, 2016 (*A. suis*, *P. multocida* and *S. suis*) and January 1, 2010 to December 31, 2016 (*H. parasuis*) was used in this study. Samples consisted on tissue sections or swabs and were cultured on tryptic soy agar with 5% sheep blood aerobically with and without 5% CO_2 for 18-24 hours for bacterial isolation except in the case of *H. parasuis* isolation, for which sheep blood agar plates were additionally supplemented with a streak of *Staphylococcus aureus* that acted as a

source of a required factor (nicotinamide adenine dinucleotide) needed for *H. parasuis* growth. Bacterial colonies were characterized at the bacterial species level by standard biochemical testing and/or MALDI-TOF mass spectrometry. Minimum inhibitory concentrations (MIC) were determined by the broth microdilution method using the Sensititre automatized dilution system (Trek Diagnostic Systems, Cleveland, Ohio). A range of dilutions was tested for a number of antimicrobials. Antimicrobial susceptibility testing of *S. suis* and *P. multocida* isolates was performed as per the standardized Clinical and Laboratory Standards Institute (CLSI) methodology.[471] MIC values for *A. suis* and *H. parasuis* were determined by growing standardized quantities of bacterial suspensions on cation-adjusted Mueller Hinton broth and *Haemophilus* test medium respectively for 18-24 hours at 35-37^0 C aerobically with 5% CO_2. Quality control was maintained following CLSI guidelines.[471]

Data extraction and statistical analysis

Antimicrobial susceptibility data, along with information on the age of the pig, date of isolation, organs from which the bacteria were isolated, and geographical state of origin were extracted from the computerized database maintained at the UMN-VDL. Because multiple isolates of the same bacterial species were collected from samples submitted by the same client, only isolates with unique MIC value-combinations for all antimicrobials were selected per submitting client for further analyses.

S. suis and *P. multocida* isolates were classified as "resistant" or "non-resistant" to specific antimicrobials using CLSI breakpoints when available (tables 4-1). For those antimicrobials for which swine specific CLSI breakpoints were not available, breakpoints applicable to other species (humans, cattle) or epidemiological cut-offs (gentamicin- *P.*

multocida) were used, if available (tables 4-2 and 4-3). There were no breakpoints or epidemiological cut-offs available for *A. suis* and *H. parasuis*. MIC_{50} and MIC_{90}, the 50^{th} and 90^{th} percentile of the MIC distributions respectively, were also estimated and presented in tables 4-2, 4-3, 4-4 and 4-5.

Changes in AMR levels over time were estimated for each bacteria-antimicrobial combination using logistic regression models. Specifically, the status of bacterial isolates (resistant or non-resistant) was used as a binary dependent variable and time (in years) as a continuous independent variable of interest as described elsewhere.[472,473] Models also accounted for the association with variables such as organ or system of isolation (respiratory or other organs such as liver, joints, heart etc.), season (winter, fall, spring and summer), and state of origin (Minnesota or elsewhere in the U.S.), which were added as categorical fixed effects. Changes in AMR prevalence over time were quantified using the exponential of the coefficient for the time variable, referred to as "time associated odds ratio" from here onwards.

For those antimicrobial- bacterial species combinations for which dichotomization of antimicrobial susceptibility results was not possible due to the lack of available breakpoints, ordinal regression models were built. In these models, the dependent variable was the MIC value of the isolates, while accounting for the association with the independent variables previously described. Because change across time was the output of interest, the results of the model outputs may be interpreted as the odds of being at a MIC value or higher as compared to any MIC value below, for a unit change in time (one year).

For these ordinal regression models, the proportional odds assumption was statistically tested[474] and if the assumption was not met, non-proportional odds ordinal logistic regression models were fitted. Due to convergence issues, MIC values ranging from 2-5 dilutions were binned together if needed. A primary difference between a proportional and non-proportional odds logistic regression models is that the former provides a single odds ratio for all levels and it is more parsimonious as compared to the latter, which provides a separate time associated odds ratio for each MIC level. The results of the abovementioned models are summarized in the tables S4-1, S4-2, S4-3 and S4-4.

Because breakpoints and epidemiological cut-offs were not available for *A. suis* and *H. parasuis*, authors have resorted to using cut-offs defined by themselves or using clinical breakpoints from bacteria of family *Pasteurellaceae* (*Actinobacillus pleuropneumoniae* and *Histophilus somni*) to analyze antimicrobial susceptibility results of *H. parasuis* (table 4-6). The impact of using different breakpoints on the interpretation of prevalence and trends in AMR data for *A. suis* and *H. parasuis* was assessed by estimating the percentage of resistant isolates and building the binary logistic regression models previously described. The agreement between AMR prevalence estimates calculated using different breakpoints were statistically evaluated using unbiased Kappa coefficient and prevalence-associated bias-associated Kappa (PABAK) coefficients.[475] PABAK coefficients greater than 0.75, between 0.50-0.74 as fair and less than 0.50 were considered to be good, fair and poor agreement.

Results

Description of data

Overall, 20,230 isolates were analyzed in this study. Nearly 66% of those isolates were either *S. suis* or *P. multocida* and 6.5 % of the isolates were *H. parasuis*. Nearly 74, 80 and 88% of the *A. suis*, *H. parasuis* and *P. multocida* isolates were obtained from the respiratory tract, whereas 90% of the *S. suis* isolates with AMR data available were collected from organ systems other than respiratory system. The majority (45%) of the isolates were collected from diagnostic samples submitted by clients in Minnesota. Isolates from the rest of the top ten pork producing states in USA made up for 45% of the isolates. Age information was available for 84% of the isolates, of which 78% were recovered from pigs 1-6 months of age.

Streptococcus suis

Briefly, breakpoints were available for 8 antimicrobials tested in >7,000 isolates, and prevalence of resistance to 6 of them was below 10% (table 4-2). In fact, the overall prevalence of resistance to five antimicrobials (ampicillin, ceftiofur, enrofloxacin, florfenicol and trimethoprim-sulphamethoxazole) was low (<3%) (table 4-2) and ranged between 0-3% across the years. The overall prevalence of penicillin resistance was 7.0% and varied from 5.3% in years 2006-09 to 9.7% in years 2013-16 (9% annual increase in odds, $p<0.01$). Levels of oxytetracycline and chlortetracycline resistance were always high and ranged between 93-97% over the years.

Breakpoints were not available for gentamicin, spectinomycin, tiamulin, tulathromycin and sulphadimethoxine and MIC distributions for these antimicrobials are

presented in table 4-2. There was a 10-17% annual increase in the odds of being at higher MIC values (≥4 µg/ml) as compared to lower MIC values for gentamicin ($p<0.01$) (figure 4-1a). For sulphadimethoxine, there was a modest 4% decrease in odds of having MIC value of >256 µg/ml over the years ($p<0.01$). For tulathromycin, there were no statistically significant annual changes in odds of having different MIC values. The results were inconclusive for spectinomycin and tiamulin, with both statistically significant annual increases and decreases in odds for some MIC values for these antimicrobials. For example, in case of spectinomycin, there was a 7% annual decrease ($p<0.01$) and 3% annual increase ($p<0.01$) in odds of having MIC values ≥32 µg/ml and ≥128 µg/ml as compared to lower MIC values, respectively.

Pasteurella multocida

Breakpoints were available for 10 antimicrobials tested in > 8000 isolates, with overall AMR prevalence being less than 10% and annual values ranging between 0 to 8% in 8 of them (ampicillin, ceftiofur, enrofloxacin, florfenicol, gentamicin, penicillin, spectinomycin and tulathromycin) (table 4-3). Resistance against the remaining two antimicrobials (oxytetracycline and chlortetracycline) varied widely and ranged between 28-56% (chlortetracycline) and 52-72% (oxytetracycline) during the study period, with logistic regression models suggesting a modest (4-6%) annual increase in the odds of resistance ($p<0.01$).

Breakpoints were not available for tiamulin, sulphadimethoxine and trimethoprim-sulphamethoxazole and MIC distributions for these are provide in table 4-3. For tiamulin, there was a statistically significant annual decrease (2-8%) in the odds of having MIC values ≥4 µg/ml as compared to lower MIC values. There was a modest

annual increase (3%) in odds of having sulphadimethoxine MIC value of >256 µg/ml ($p<0.01$). The odds of having MIC values (>2 µg/ml) did not change significantly for trimethoprim-sulphamethoxazole ($p=0.06$).

Actinobacillus suis

There were no breakpoints available for any of the antimicrobials for *A. suis* and detailed MIC distributions are presented in table 4-4. There was no statistically significant change in the odds of having different MIC values for enrofloxacin, trimethoprim-sulphamethoxazole and gentamicin. There was a statistically significant annual decrease of 15-29% in the odds of having higher MIC values for ceftiofur (≥0.5 µg/ml), spectinomycin (≥16 µg/ml), sulphadimethoxine (>256 µg/ml) and tiamulin (≥1 µg/ml) as compared to lower MIC values (figures 4-1b, c, d). For ampicillin, there was a statistically significant ($p<0.05$) yet modest decrease (4-7%) in the odds of being at MIC values ≥0.5 µg/ml as compared to baseline MIC value of 0.25 µg/ml. The change in odds of being at different MIC levels were less uniform and inconclusive for oxytetracycline, florfenicol, tulathromycin and penicillin compared to other antimicrobials, with either a statistically significant increase or decrease at some MIC levels but non-significant changes at other MIC values. Similarly, the change in odds for chlortetracycline were ambiguous and inconclusive, with a 4% annual increase at MIC value ≥1 µg/ml but a 5-7% annual decrease at MIC values ≥4 µg/ml as compared to lower MIC values.

Haemophilus parasuis

There were no breakpoints available for any of the antimicrobials for *A. suis* and detailed MIC distributions are presented in table 4-5. There was no significant change in the odds of having different MIC values for chlortetracycline, tiamulin, trimethoprim-

sulphamethoxazole and spectinomycin. However, there was a consistent annual increase of 9-20% in the odds of having higher MIC levels for ampicillin (≥0.5 µg/ml), florfenicol (≥0.5 µg/ml), gentamicin (≥2 µg/ml) and penicillin (≥0.25 µg/ml) as compared to lower MIC values ($p<0.01$) (figures 4-1e, f, g, h). This annual increase was even higher for ceftiofur (≥0.5 µg/ml), enrofloxacin (≥0.25 µg/ml), and tulathromycin (≥2 µg/ml) MIC values as compared to lower MIC values, ranging between 23-34% ($p<0.01$) (figures 4-1 i, j, k). There was a 12% annual decrease in odds of being at high MIC values for sulphadimethoxine (>256 µg/ml) ($p<0.01$). The annual change in odds of having different MIC values were inconclusive for oxytetracycline, with a statistically significant decrease in annual odds at one MIC level but non-significant changes in odds for other MIC values.

Impact of the use of different breakpoints on interpretation of AMR data in A. suis and H. parasuis

Overall, the effect of using different breakpoints for interpretation of AMR data on the prevalence of resistance was evident in both bacterial species for several antimicrobials assessed (tables 4-6 and 4-7). Breakpoints used in the estimations ranged from 1-3 dilution levels for the different antimicrobial-bacterial species combinations. The effect of using different breakpoints on the estimated annual changes was often negligible and confidence intervals of estimates for the same bacteria-antimicrobial combination but using different breakpoints were overlapping. However, there were differences in agreement between AMR prevalence estimates calculated using different breakpoints (table 4-7). For *A. suis*, PABAK coefficients were good, fair and poor for 5, 1 and 2 out

of 8 pairs of breakpoints compared. For *H. parasuis*, PABAK coefficents were good and fair for 4 pairs of breakpoints each.

Discussion

The SRDC causes huge economic losses and increased mortality in swine herds worldwide. Antimicrobials are one of the main tools for control of SRDC, but there is limited available information on the prevalence of AMR in these bacteria in comparison with other zoonotic pathogens such as *Salmonella* or *Campylobacter* (NARMS), even though this information could help to guide therapy and detect changes over time. In this study, we estimated the prevalence of AMR against a panel of 13 antimicrobials and the changes in the levels of resistance over a decade in key bacterial pathogens involved in SRDC in the U.S. using data collected at the UMN-VDL, a NARMS laboratory that processes a large number of swine samples in the country.

For *S. suis*, resistance levels determined in swine isolates from Europe for ampicillin, ceftiofur, enrofloxacin and florfenicol resistance were similar to the results reported here (<3%), whereas higher levels, compared to Europe, were observed for trimethoprim-sulphamethoxazole (3-12% compared with 2% here).[116,476–480] Reports on penicillin resistance levels in Europe are much more heterogeneous (ranging from <2% to >21%)[477,478,480] but in some cases were in agreement with values described here (7%).[476,479] With a few exceptions, European studies also reported tetracycline resistance levels above 80% in *S. suis* isolates.[116,476–480] For those antimicrobials with no available breakpoints (gentamicin, spectinomycin, tiamulin and tulathromycin) the estimated MIC_{50} and MIC_{90} values were within one dilution level of those reported previously in Europe,[116,476,478–480] with the only exception of a lower MIC_{90} value (16 µg/ml) for

spectinomycin[478] and higher MIC_{50} (32 µg/ml) and MIC_{90} (128 µg/ml) values for tiamulin[477] compared to the findings of this study.

Outside Europe reports of the prevalence of AMR in *S. suis* are highly variable. Prevalence levels similar to those reported here were also reported for ampicillin, enrofloxacin, penicillin, tetracycline and trimethoprim-sulphamethoxazole resistance in Australian *S. suis* isolates, while higher levels of florfenicol resistance were observed (15%) compared to our results.[481] In stark contrast, Chinese *S. suis* were found to be highly resistant to clindamycin (98%), ceftiofur (56%) and penicillin (75%).[482]

AMR prevalence to ceftiofur, enrofloxacin, florfenicol, tulathromycin and trimethoprim-sulphamethoxazole and MIC_{50} and MIC_{90} values for tiamulin and spectinomycin in European and Australian *P. multocida* isolates were similar to values found here.[116,483] However, both Dayao et al. (2014) and El Garch et al. (2016) found that 20-28% of the *P. multocida* isolates were tetracycline resistant, whereas 40-60% of *P. multocida* isolates in this study were chlortetracycline and/or oxytetracycline resistant.[116,483] In contrast, the prevalence of resistance to antimicrobials (spectinomycin, chlortetracycline, trimethoprim-sulphamethoxazole), MIC_{50}, and MIC_{90} values (gentamicin) reported from clinical swine isolates in China was higher with the exception of ceftiofur and florfenicol resistance (Tang et al., 2009).[484] Similarly, in isolates from diseased pigs in Taiwan, prevalence of enrofloxacin (61%), florfenicol (92%), and tiamulin (MIC_{50} – 128 µg/ml, MIC_{90}- >128 µg/ml) resistance was comparatively higher.[485]

In absence of clinical breakpoints and epidemiological cut-offs, MIC distributions are an alternative source of information on AMR levels.[486] For *H. parasuis*, MIC_{50} and

MIC_{90} values for ampicillin, ceftiofur, florfenicol, gentamicin, penicillin, tetracycline, tulathromycin and tiamulin reported here were at least three dilution levels higher compared with values reported in European studies.[116,487] Dayao et al. (2014) estimated MIC_{90} values similar (florfenicol, oxytetracycline) or 4-7 double dilutions lower (ampicillin, ceftiofur, penicillin) in Australian *H. parasuis* isolates as compared to this study.[488] In contrast, MIC_{90} values estimated by Zhao et al. (2018) in Chinese *H. parasuis* isolates for ceftiofur, enrofloxacin, florfenicol, gentamicin and tetracycline were 2-7 dilution steps higher as compared to isolates in this study.[489] To the best of our knowledge, this is the first study to publish MIC distributions for *A. suis* and hence, results comparison to earlier studies was not possible.

Significant changes in the MIC distributions of some bacterial-antimicrobial combinations over the years were observed in this study. Notable changes included increases >10% in odds of being at higher MIC values for gentamicin and tiamulin (*S. suis*) and 7 antimicrobials in *H. parasuis* isolates; However, decreases >10% in the odds of being at higher MIC values for 5 antimicrobials in *A. suis* and sulphadimethoxine in *H. parasuis* isolates were also reported. Nevertheless, for bacterial-antimicrobial combinations for which breakpoints were available, the prevalence of resistance never exceeded 10%, even though some trends were statistically significant (tables S4-1, S4-2, S4-3 and S4-4). Similarly, El Garch et al. (2016) reported no change in AMR in European *S. suis* and *P. multocida* isolates, except for a significant increase in ceftiofur resistance and a non-significant increase to tetracycline resistance in *S. suis.* A drastic increase in resistance to spectinomycin and tiamulin and non-significant changes in

resistance to other antimicrobials in *S. suis* isolates was also reported by Hernandez-Garcia et al. (2016).[476]

The interpretation of AMR data is severely hindered due to lack of host-bacteria-antimicrobial specific clinical breakpoints.[116] Moreover, we have shown here that the use of different breakpoints may have a significant impact on the estimates of prevalence of resistance and thus, breakpoints from related bacteria should be used cautiously for interpretive purposes. Cusack et al. (2018) re-analyzed 20 AMR studies using CLSI and EUCAST breakpoints for *Enterobacteriaceae* and observed significant discrepancies in prevalence estimates in 19 of them.[490] Although we estimated that the odds of being at higher MIC levels for several antimicrobials were changing significantly, the clinical or epidemiological interpretation of these results can be contentious due to lack of interpretive criteria. Moreover, the methodology of susceptibility testing of *H. parasuis* and *A. suis* has not been standardized by CLSI or EUCAST.[487,491] Hence, there is a clear need to establish methodology, epidemiological cut-offs and clinical breakpoints for bacteria relevant to animal health to make clinical and epidemiological references.[470]

There are some limitations that should be considered in the context of this study. First, information on the serotype or presence of virulence factors was not available for any bacteria, and for instance serotype distribution has been associated with AMR in *S. suis* and *H. parasuis* (Yeh et al., 2016; Yongkiettrakul et al., 2019).[485,492] Second, the information on antimicrobial use in the farm prior to collection of the analyzed samples was not available. Third, the isolates were mostly from Minnesota and might not be representative of true prevalence of AMR in these swine pathogens in the whole U.S.

However, incorporating geographical information in statistical models did not lead to significant changes in the estimates.

Conclusions

We described the prevalence and changes of AMR in some key bacterial pathogens of great importance to swine medicine. Results described here will help in surveillance of AMR in these critical swine pathogens and aid in informed decision-making regarding antimicrobial use in swine medicine. Future research work should focus on continuing and strengthening AMR surveillance in these pathogens, as well as establishing standardized methods to test AMR in *A. suis* and *H. parasuis* and epidemiological cut-offs and clinical breakpoints for specific bacterial species so that results can be harmonized globally. Additionally, genomic characterization and correlating MIC values with presence/absence of AMR genes in these pathogens can aid in allowing microbiological/genomic inferences to these results in addition to statistical modelling.

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Tables

Table 4-1. Breakpoints* (in μg/ml) used for classifying isolates as resistant

Antimicrobial	*Streptococcus suis*	*Pasteurella multocida*	*Actinobacillus suis*	*Haemophilus parasuis*
Ampicillin	2	2	NA	NA
Ceftiofur	8	8	NA	NA
Chlortetracycline	2	2	NA	NA
Enrofloxacin	2	1	NA	NA
Florfenicol	8	8	NA	NA
Gentamicin	NA	16	NA	NA
Oxytetracycline	2	2	NA	NA
Penicillin	1	1	NA	NA
Spectinomycin	NA	>64	NA	NA
Sulphadimethoxine	NA	NA	NA	NA
Tiamulin	NA	NA	NA	NA
Trimethoprim-Sulphamethoxazole	4	NA	NA	NA
Tulathromycin	NA	64	NA	NA

*- Isolates with MIC values equal to or more than these breakpoints were considered to be “resistant”.

Table 4-2. MIC distribution frequencies of *Streptococcus suis* isolates collected at UMN-VDL from 2006-2016.

Antimicrobial (n)*	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	% resistance	MIC_{50}	MIC_{90}
Ampicillin (7010)		98.0	1.0	0.3	0.2	0.2	0.1	0.2						0.6	≤0.25	≤0.25
Ceftiofur (7025)			93.7	3.2	1.4	0.7	0.5	0.5						1.0	≤0.5	≤0.5
Chlortetracycline (7011)				1.0	0.7	2.0	13.4	78.8						94.9	>8	>8
Enrofloxacin (6377)	6.3	35.3	51.0	5.9	0.8	0.7								1.5	0.5	0.5
Florfenicol (7002)		0.9	3.4	19.9	68.6	6.2	0.7	0.3						1.0	2	2
Gentamicin (7025)				8.0	27.6	46.4	15.0	1.5	1.5					-	4	8
Oxytetracycline (7025)			2.9	1.7	0.7	1.1	4.3	89.3						95.4	>8	>8
Penicillin (7025)	86.5	3.3	3.2	2.6	2.8	0.9	0.4	0.3						7.0	≤0.12	0.5
Spectinomycin (7025)							6.7	51.3	29.0	2.8	10.2			-	16	>64
Sulphadimethoxine (7016)												41.2	58.8	-		

Tiamulin (6999)			23.3	42.3	15.5	2.7	1.6	2.9	4.6	7.1				-	1	32
Trimethoprim-Sulphamethoxazole (7016)					98.0	2.0								2		
Tulathromycin (5207)				7.6	13.4	7.5	1.1	0.7	1.5	2.8	65.4			-	>64	>64

Shaded areas indicate concentrations not tested. Red lines demarcate resistant and not-resistant isolates based on swine-specific breakpoints (CLSI, 2018) except trimethoprim-sulphamethoxazole (human-specific breakpoints). *- number of isolates tested for susceptibility to this antimicrobial.

Table 4-3. MIC distribution frequencies of *Pasteurella multocida* isolates collected at UMN-VDL from 2006-2016.

Antimicrobial (n)*	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	% resistance	MIC50	MIC90
Ampicillin (8667)		85.0	8.5	2.4	1.2	0.6	0.4	0.4	1.6					4.2	≤0.25	0.5
Ceftiofur (8670)			96.9	1.3	0.4	0.3	0.4	0.7						1.0	≤0.5	≤0.5
Chlortetracycline (8670)			33.2	26.4	23.5	12.0	3.4	1.5						40.4	1	4

Enrofloxacin (7684)	93.9	3.2	2.1	0.4	0.2	0.2								0.8	≤0.12	≤0.12
Florfenicol (8670)		21.9	59.6	11.3	5.5	0.6	0.3	0.8						1.1	0.5	1
Gentamicin (8671)				36.8	48.5	11.3	1.6	0.9	0.9					1.8	2	4
Oxytetracycline (8671)			24.0	13.8	26.3	8.9	2.7	24.3						62.2	2	>8
Penicillin (8671)	77.6	14.7	2.8	1.4	0.9	0.4	0.5	1.7						4.8	≤0.12	0.25
Spectinomycin (8671)							15.1	32.7	43.3	6.7	2.1			2.1	32	32
Sulphadimethoxine (8665)												28.5	71.5	-		
Tiamulin (8666)						2.2	8.7	31.7	43.3	14.1				-	32	>32
Trimethoprim-Sulphamethoxazole (8665)					95.9	4.1								-		
Tulathromycin (7318)				55.3	25.6	12.1	3.6	0.8	0.7	0.7	1.3			2.0	≤1	4

Shaded areas indicate concentrations not tested. Red lines demarcate resistant and not-resistant isolates based on swine-specific breakpoints (CLSI, 2018) except spectinomycin (cattle-specific breakpoints). *- number of isolates tested for susceptibility to this antimicrobial.

Table 4-4. MIC distribution frequencies of *Actinobacillus suis* isolates collected at UMN-VDL from 2006-2016.

Antimicrobial (n)*	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	MIC_{50}	MIC_{90}
Ampicillin (2862)		83.5	9.3	1.5	0.7	0.6	0.7	0.9	2.9					≤0.25	0.5
Ceftiofur (2564)		93.7	2.7	1.5	1.2	0.5	0.1	0.2						≤0.25	≤0.25
Chlortetracycline (2865)			37.8	30.1	8.8	13.6	7.6	2.0						1	4
Enrofloxacin (2610)	94.4	3.0	1.6	0.5	0.3	0.2								0.12	0.12
Florfenicol (2865)		8.5	72.1	15.3	3.2	0.3	0.1	0.5						0.5	1
Gentamicin (2866)				61.2	33.1	3.7	0.7	0.6	0.6					≤1	2
Oxytetracycline (2865)			49.1	20.1	0.9	0.5	1.7	27.7						1	>8
Penicillin (2866)	6.1	10.7	52.6	23.0	2.5	0.3	0.5	4.3						0.5	1

Spectinomycin (2865)							2.4	14.3	76.0	5.2	2.2			32	32
Sulphadimethoxine (2864)												79.7	20.3		
Tiamulin (2859)			0.2	0.1	0.2	1.2	42.1	50.6	4.6	0.8				16	16
Trimethoprim-Sulphamethoxazole (2864)					98.6	1.4									
Tulathromycin (2432)				20.8	61.4	11.3	2.6	0.5	0.3	0.7	2.4			2	4

Shaded areas indicate concentrations not tested.

*- number of isolates tested for susceptibility to this antimicrobial.

Table 4-5. MIC distribution frequencies of *Haemophilus parasuis* isolates collected at UMN-VDL from 2010-2016.

Antimicrobial (n)*	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	MIC_{50}	MIC_{90}
Ampicillin (1658)		46.8	9.8	11.3	10.2	7.4	2.1	0.9	11.5					0.5	>16
Ceftiofur (1658)		82.8	5.6	3.6	1.3	0.7	0.4	5.7						≤0.25	1
Chlortetracycline (1658)			82.1	9.2	6.6	1.2	0.6	0.2						≤0.5	1
Enrofloxacin (1658)	94.8	2.8	1.6	0.4	0.2	0.3								≤0.12	≤0.12
Florfenicol (1658)		58.5	34.4	5.5	1.3	0.1	0.1	0.1						≤0.25	0.5
Gentamicin (1658)				22.9	24.0	33.4	15.9	3.0	0.8					4	8
Oxytetracycline (1658)			53.3	15.7	13.0	5.1	4.5	8.4						≤0.5	8
Penicillin (1658)	38.7	17.4	14.8	10.8	5.9	1.1	1.1	10.1						0.25	>8
Spectinomycin (1658)							73.8	12.8	7.4	3.9	2.1			≤8.0	32.0
Sulphadimethoxine (1658)												45.4	54.6		
Tiamulin (1658)			7.7	12.2	21.5	27.7	22.0	7.5	1.0	0.5				4	8

Trimethoprim-Sulphamethoxazole (1658)					86.1	13.9									
Tulathromycin (1658)				21.5	16.9	20.8	24.0	9.3	4.2	1.6	1.8			4	16

Shaded areas indicate concentrations not tested.

*- number of isolates tested for susceptibility to this antimicrobial.

Table 4-6. Impact of using different breakpoints on estimation of prevalence of AMR and changes in AMR over time.

		Actinobacillus suis			*Haemophilus parasuis*		
Antimicrobial	Breakpoint (in µg/ml)	Odds ratio* (95% CI)	p-value	Percentage of AMR (in %)	Odds ratio* (95% CI)	p-value	Percentage of AMR (in %)
Ampicillin	2[a]	0.96 (0.91 - 1.01)	0.10	5.73	1.18 (1.12 - 1.25)	<0.01	32.1
	4[bc]	0.95 (0.90 -1.0)	0.05	5.07	1.23 (1.15 - 1.31)	<0.01	21.9
	8[d]	0.94 (0.89 -1.0)	0.04	4.51	1.25 (1.16 - 1.34)	<0.01	14.5
Chlortetracycline	2[acef]	0.94 (0.91	<0.01	32.0	1.04 (0.95	0.43	8.69

		- 0.95)			- 1.14)		
	8[bg]	0.95 (0.92 - 0.99)	0.02	9.16	1.14 (0.87 - 1.49)	0.33	0.84
	16[d]	1.02 (0.94 - 1.10)	0.67	2.02	1.39 (0.87 - 2.24)	0.17	0.24
Enrofloxacin	1[aef]	1.03 (0.91 - 1.16)	0.67	1.03	1.38 (1.07 - 1.79)	0.01	0.84
	2[bdg]	0.87 (0.73 - 1.03)	0.11	0.54	1.85 (1.27 - 2.72)	<0.0 1	0.48
Gentamicin	8[a]	0.99 (0.91 - 1.07)	0.78	1.95	1.17 (1.10 - 1.25)	<0.0 1	19.7

	16[bd]	0.97 (0.87 - 1.07)	0.54	1.26	1.22 (1.08 - 1.39)	<0.01	3.80
Penicillin	1[eg]	0.97 (0.95 -1.0)	0.03	30.6	1.16 (1.10 - 1.23)	<0.01	29.1
	4[bcd]	0.93 (0.88 - 0.98)	0.01	5.09	1.22 (1.13 - 1.31)	<0.01	12.4
Tulathromycin	16[aeg]	0.96 (0.89 - 1.04)	0.30	3.82	1.14 (1.07 - 1.22)	<0.01	16.8
	64[c]	0.94 (0.87 - 1.02)	0.17	3.09	1.28 (1.12 - 1.46)	<0.01	3.38

[*]- Time associated odds ratio obtained by using year as a continuous independent variable in binary logistic regression models using these breakpoints.

[a]- CLSI (2018) breakpoints for *Actinobacillus pleuropneumoniae*[471]

[b]- Zhou et al. (2010)[493]

[c]- Dayao et al. (2014)[488]

[d]- de la Fuente et al. (2007)[494]

[e]- Kucerova et al. (2011)[495]

[f]- El Garch et al. (2016)[116]

[g]- CLSI (2018) breakpoints for *Histophilus somni*[471]

Table 4-7. Agreement between prevalence estimates of antimicrobial resistances after using different breakpoints based on unbiased Kappa and prevalence-associated bias-associated Kappa coefficients (PABAK)

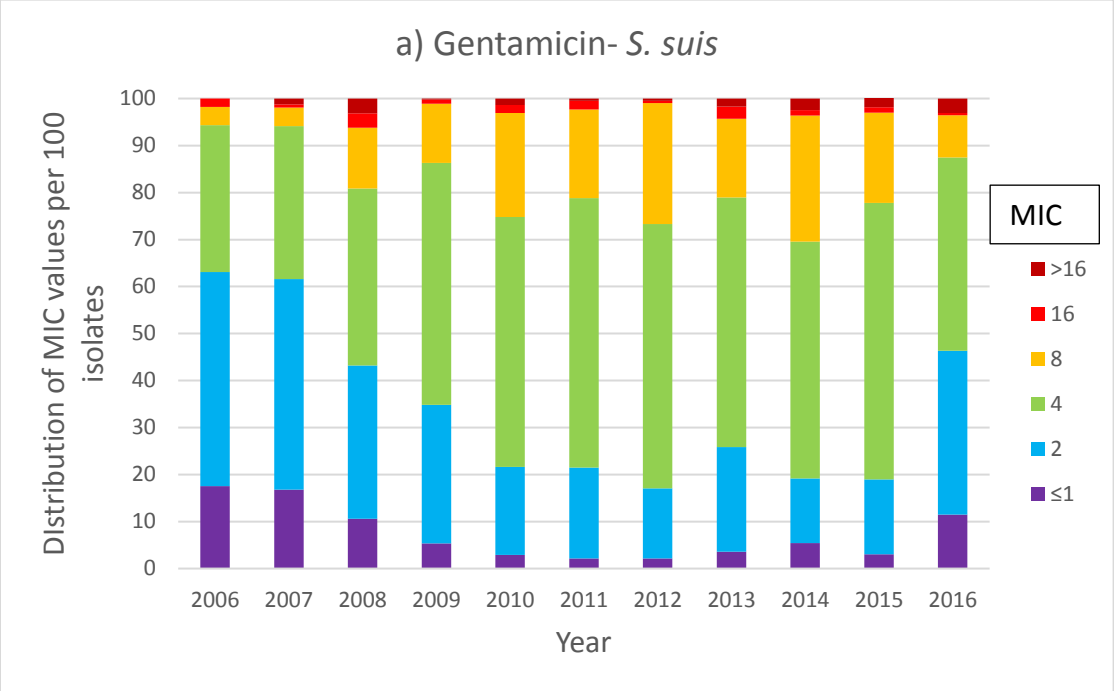
Antimicrobials (breakpoints* compared)	*Actinobacillus suis*		*Haemophilus parasuis*	
	Unbiased Kappa coefficient	PABAK coefficient	Unbiased Kappa coefficient	PABAK coefficient
Ampicillin (2 vs 4)	0.94	0.99	0.75	0.80
Ampicillin (2 vs 8)	0.87	0.98	0.55	0.66
Chlortetracycline (2 vs 8)	0.61	0.63	0.16	0.84
Chlortetracycline (2 vs 16)	0.08	0.4	0.04	0.83
Enrofloxacin (1 vs 2)	0.66	0.98	0.76	0.99

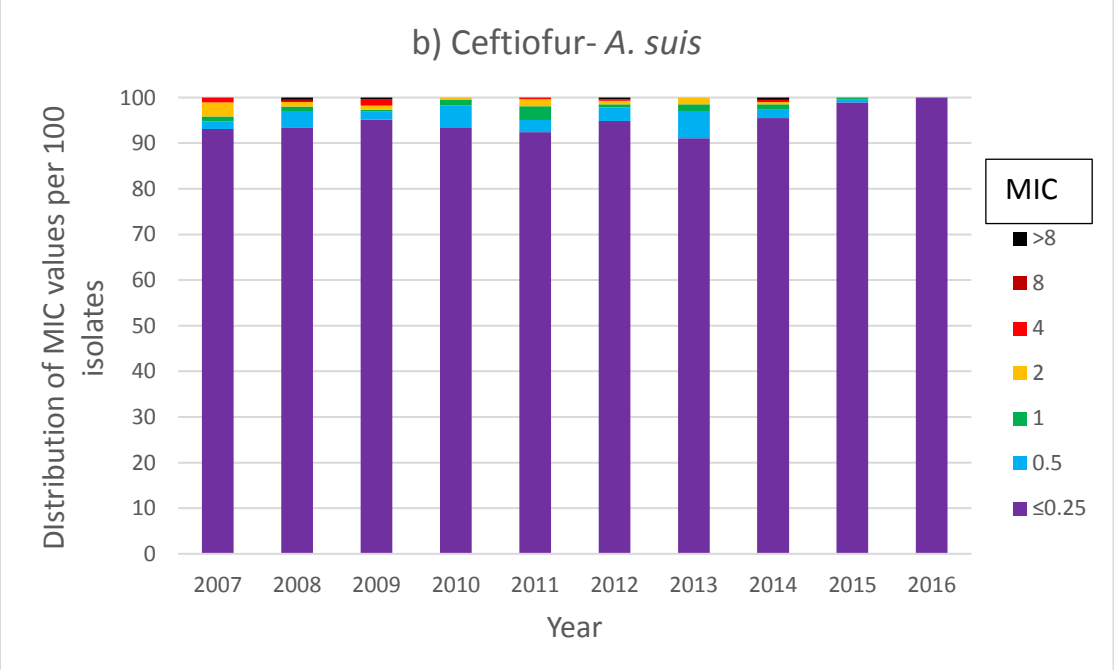
Gentamicin (8 vs 16)	0.78	0.99	0.28	0.68
Penicillin (1vs 4)	0.21	0.48	0.51	0.67
Tulathromycin (16 vs 64)	0.90	0.99	0.29	0.73

*- Breakpoints were based on MIC values (in μg/ml)

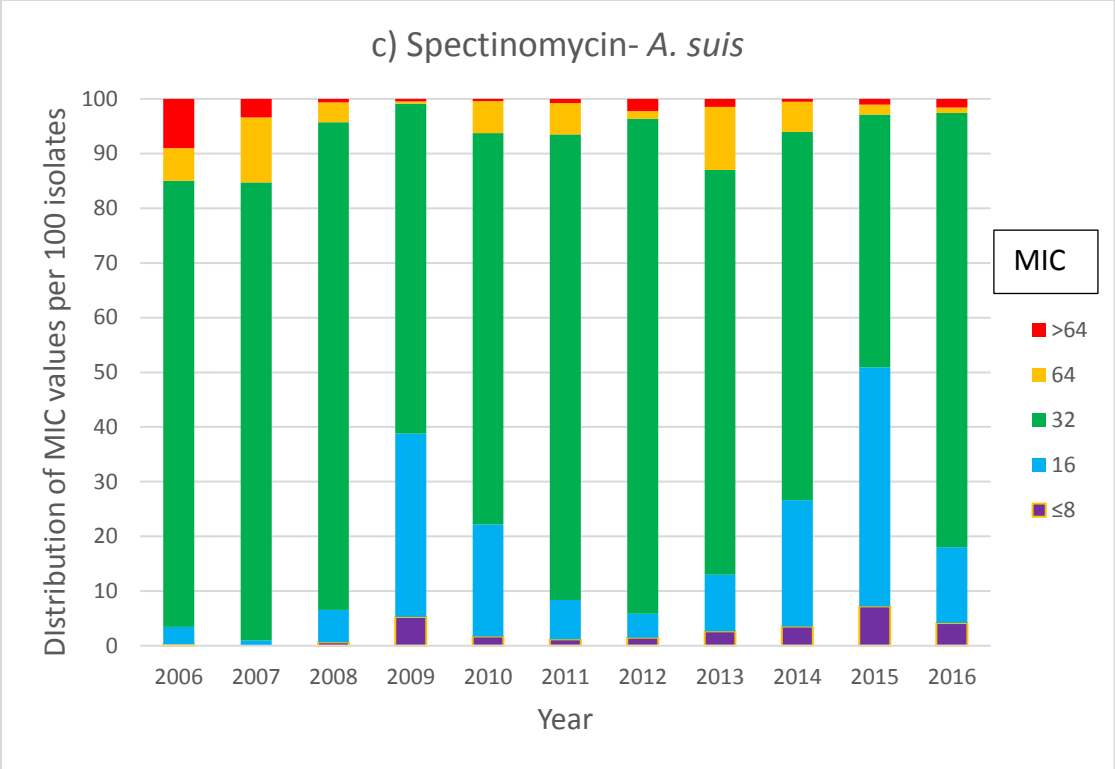
Figures

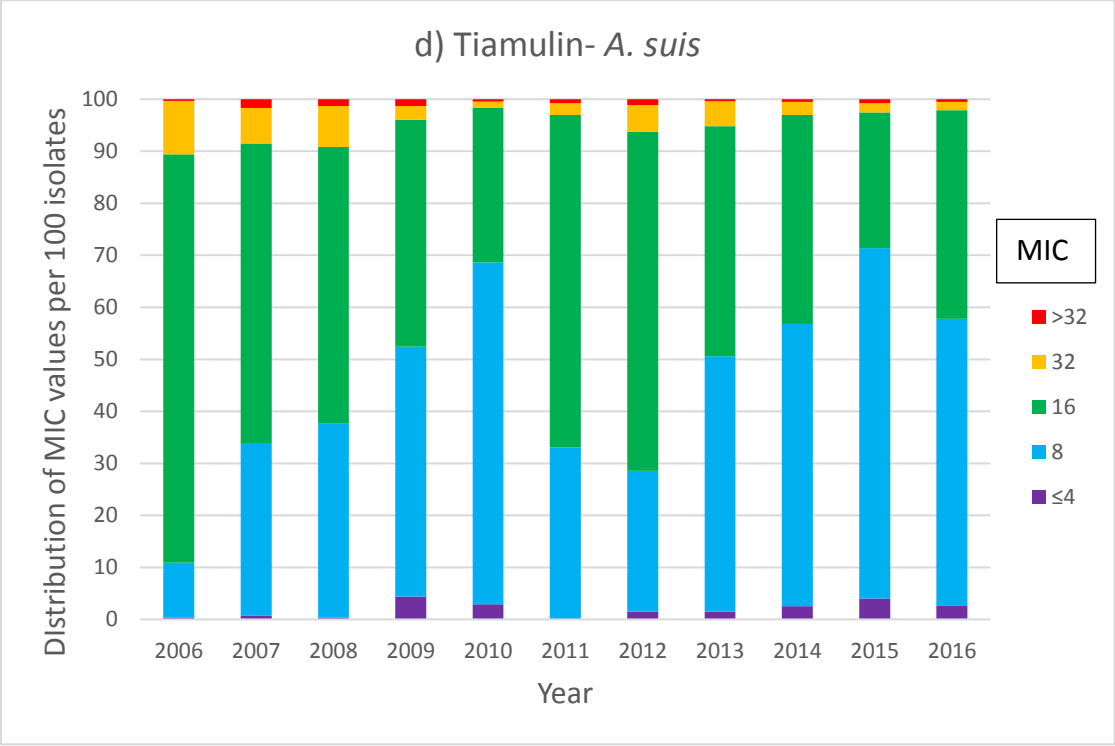
Figure 4-1. Changes in MIC distributions over years for select bacterial-antimicrobial combinations.



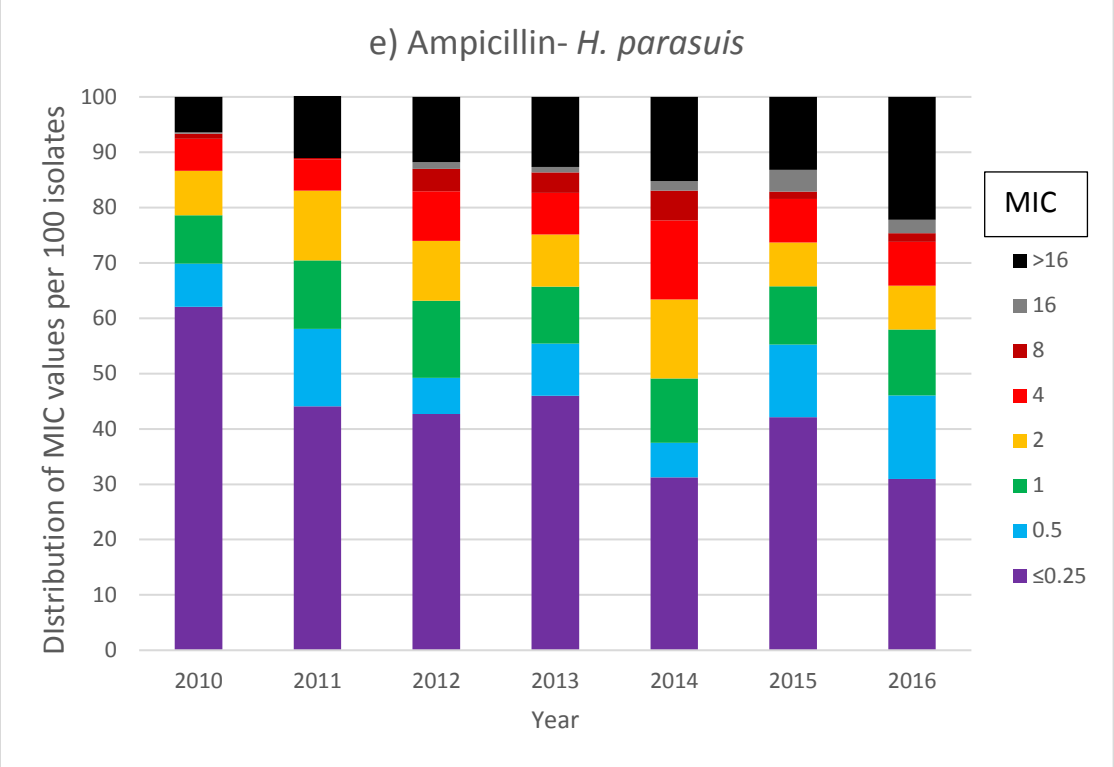


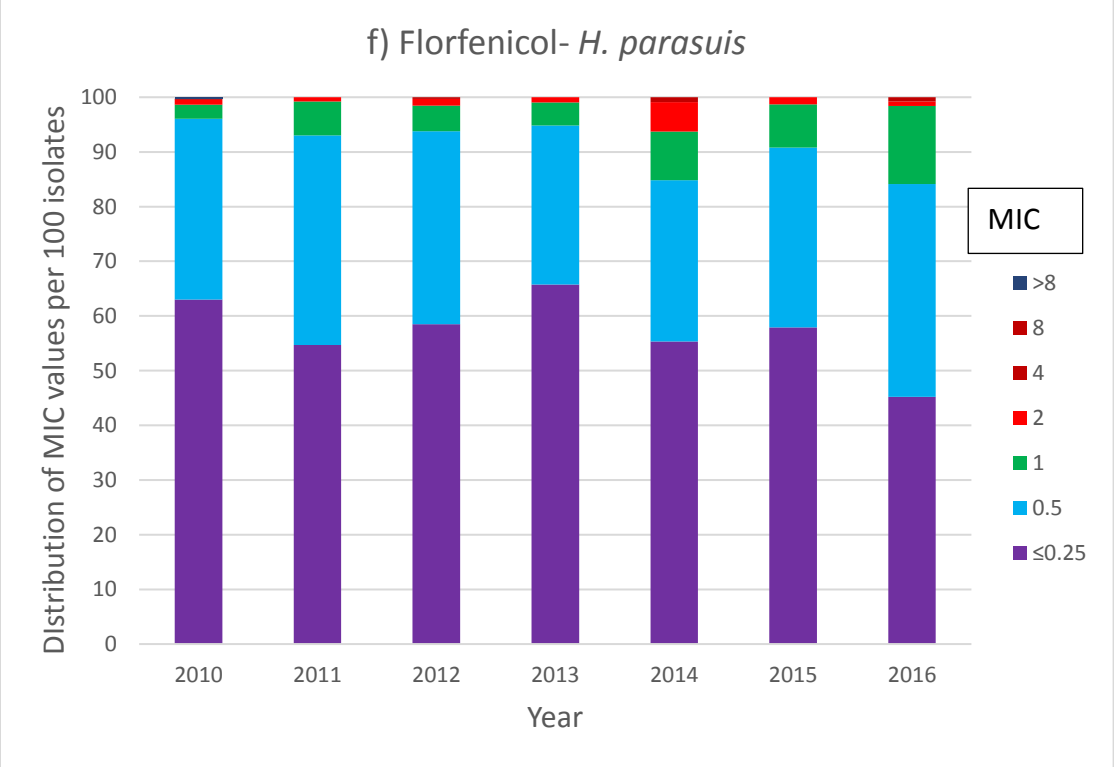
"MIC" represents minimum inhibitory combinations in µg/ml.



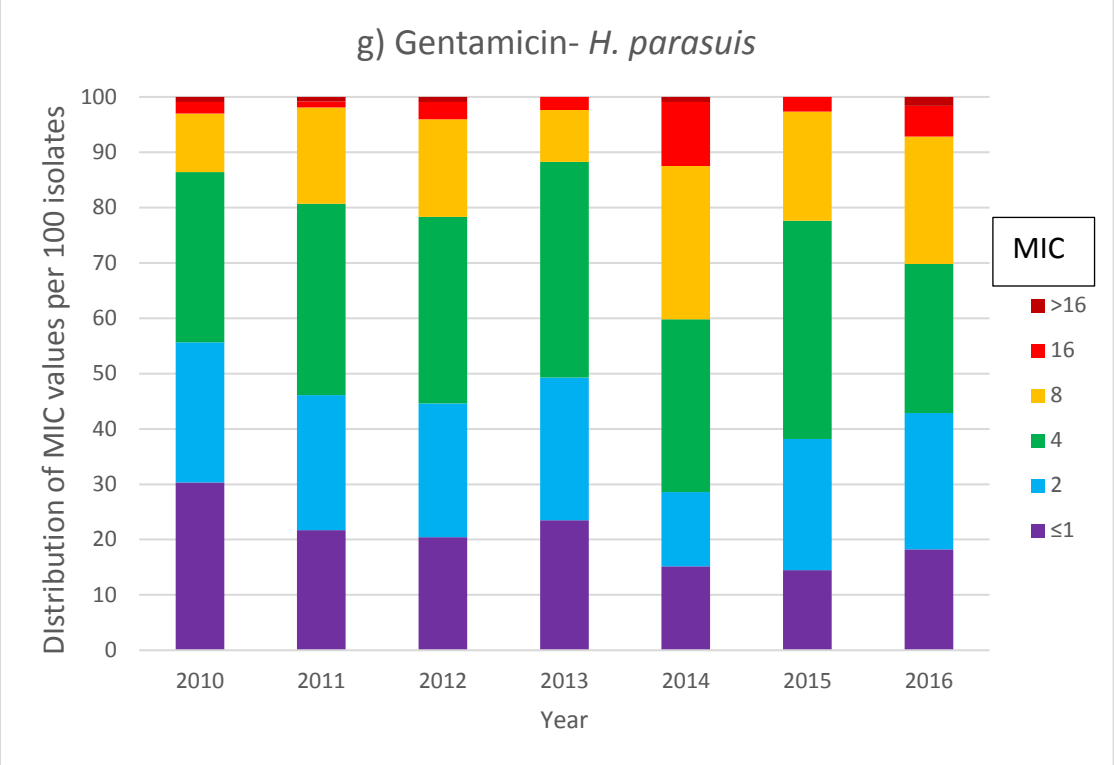


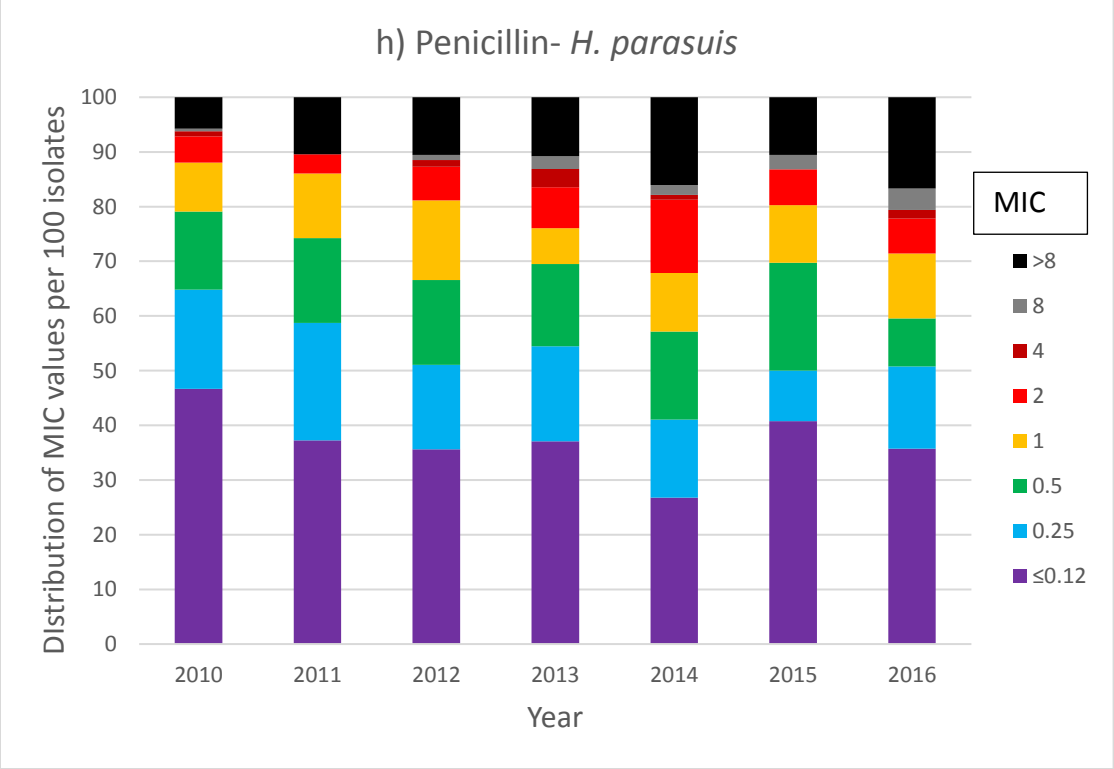
"MIC" represents minimum inhibitory combinations in µg/ml.



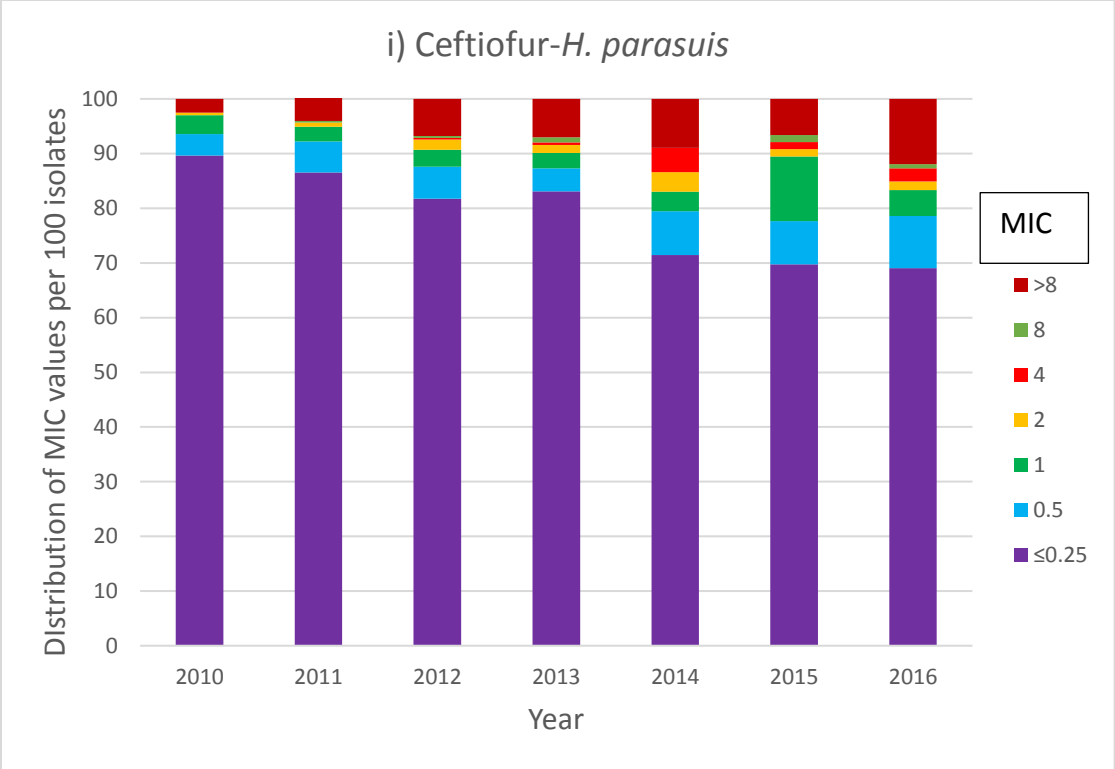


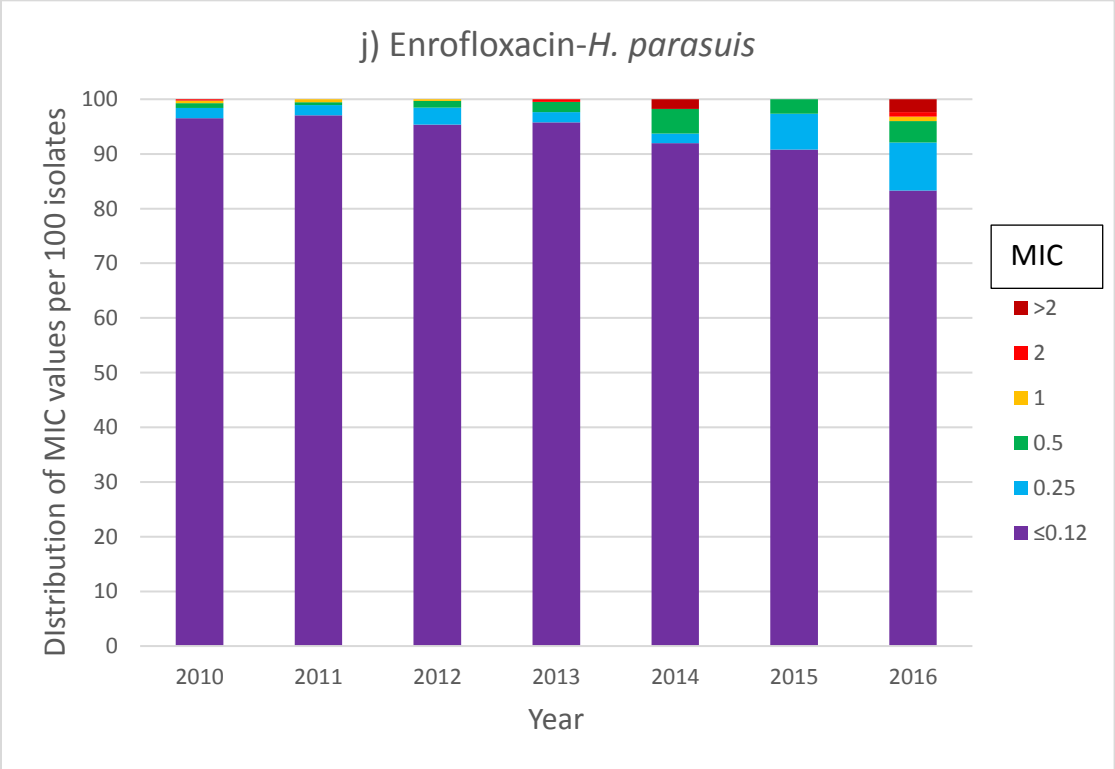
"MIC" represents minimum inhibitory combinations in µg/ml.



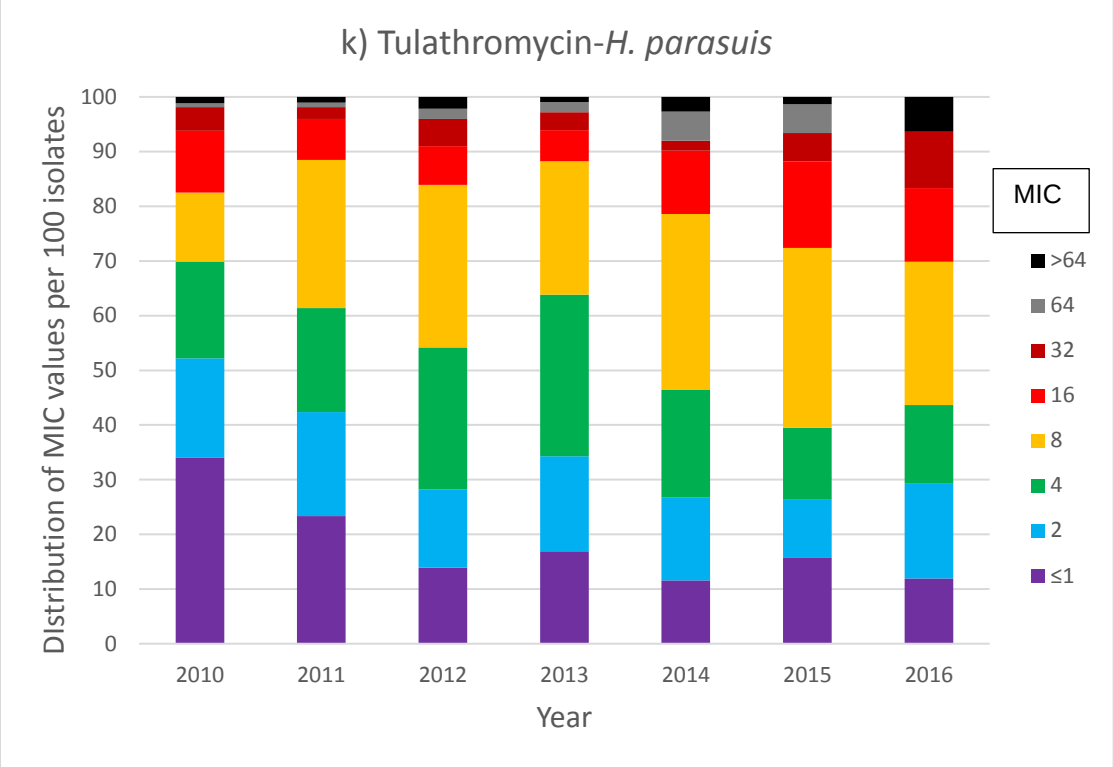


"MIC" represents minimum inhibitory combinations in µg/ml.





"MIC" represents minimum inhibitory combinations in μg/ml.



"MIC" represents minimum inhibitory combinations in μg/ml.

Supplementary materials

Table S4-1. Changes in prevalence of antimicrobial resistance in *Streptococcus suis* isolates

Antimicrobial	Proportional odds assumption met	Reference MIC values vs higher MIC values (in μg/ml)	Time associated odds ratio	p-value
Ampicillin	Breakpoint available	-	1.06 (0.97-1.16)	0.23
Ceftiofur	Breakpoint available	-	1.19 (1.10-1.29)	<0.01
Chlortetracycline	Breakpoint available	-	1.0 (0.97-1.03)	0.99
Enrofloxacin	Breakpoint available	-	0.96 (0.90-1.02)	0.19
Florfenicol	Breakpoint available	-	0.89 (0.82-0.96)	<0.01
Oxytetracycline	Breakpoint available	-	1.01 (0.97-1.04)	0.74
Penicillin	Breakpoint available	-	1.08 (1.05-1.10)	<0.01

Sulphadimethoxine		≤ 256 vs > 256	0.96 (0.94-0.97)	<0.01
Trimethoprim-Sulphamethoxazole	Breakpoint available	-	0.98 (0.93-1.04)	0.5
Gentamicin	No	≤ 2 vs ≥ 4	1.16 (1.15-1.18)	<0.01
		≤ 4 vs ≥ 8	1.10 (1.08-1.13)	<0.01
Spectinomycin	No	≤ 8 vs ≥ 16	1.05 (1.02-1.08)	<0.01
		≤ 16 vs ≥ 32	0.93 (0.92-0.94)	<0.01
		≤ 32 vs ≥ 64	1.01 (0.99 - 1.04)	0.11
		≤ 64 vs ≥ 128	1.03 (1.0-1.05)	<0.01
Tiamulin	No	≤ 0.5 vs ≥ 1	1.01 (0.89-1.15)	0.25
		≤1 vs ≥ 2	0.90 (0.89-0.91)	<0.01
		≤ 4 vs ≥ 8	1.05 (1.03-1.07)	<0.01
Tulathromycin	No	≤1 vs ≥ 2	1.07 (1.03-1.12)	<0.01
		≤ 2 vs ≥ 4	1.01 (0.99-1.04)	0.37
		≤ 4 vs ≥ 8	1.0 (0.98-1.03)	0.83
		≤ 64 vs ≥ 128	1.0 (0.98-1.02)	0.93

Table S4-2. Changes in prevalence of antimicrobial resistance in *Pasteurella multocida* isolates

Antimicrobial	Proportional odds assumption met	Reference MIC values vs higher MIC values (in μg/ml)	Time associated odds ratio	p-value
Ampicillin	Breakpoint available	-	0.93 (0.90-0.96)	<0.01
Ceftiofur	Breakpoint available	-	0.82 (0.75-0.88)	<0.01
Chlortetracycline	Breakpoint available	-	1.06 (1.05-1.08)	<0.01
Enrofloxacin	Breakpoint available	-	0.98 (0.90-1.06)	0.56
Florfenicol	Breakpoint available	-	1.06 (0.99-1.13)	0.09
Gentamicin	Breakpoint available	-	1.02 (0.97-1.07)	0.49
Oxytetracycline	Breakpoint available	-	1.04 (1.03-1.05)	<0.01

Penicillin	Breakpoint available	-	0.95 (0.92-0.98)	<0.01
Spectinomycin	Breakpoint available	-	0.90 (0.85-0.94)	<0.01
Sulphadimethoxine	-	≤ 256 vs > 256	1.03 (1.02-1.05)	<0.01
Trimethoprim-Sulphamethoxazole	-	≤ 2 vs > 2	0.97 (0.94-1.01)	0.06
Tulathromycin	Breakpoint available	-	1.0 (0.95-1.06)	0.9
Tiamulin	No	≤ 4 vs ≥ 8	0.92 (0.88-0.96)	<0.01
		≤ 8 vs ≥ 16	0.96 (0.94-0.97)	<0.01
		≤ 16 vs ≥ 32	0.98 (0.96-0.99)	<0.01
		≤ 32 vs ≥ 64	0.97 (0.95-0.99)	<0.01

Table S4-3. Changes in prevalence of antimicrobial resistance in *Actinobacillus suis* isolates

Antimicrobial	Proportional odds assumption met	Reference MIC values vs higher MIC values (in μg/ml)	Time associated odds ratio	p-value
Ampicillin	Yes	-	0.96 (0.93-0.99)	<0.01
Ceftiofur	No	≤ 0.25 vs ≥ 0.5	0.82 (0.77-0.87)	<0.01
		≤1 vs ≥ 2	0.71 (0.64-0.79)	<0.01
Chlortetracycline	No	≤ 0.5 vs ≥ 1	1.04 (1.02-1.07)	<0.01
		≤ 2 vs ≥ 4	0.93 (0.90-0.96)	<0.01
		≤ 4 vs ≥ 8	0.95 (0.91-0.99)	<0.01
Enrofloxacin	Yes	-	1.05 (0.99-1.11)	0.05
Florfenicol	No	≤ 0.5 vs ≥ 1	0.92 (0.89-0.95)	<0.01
		≤1 vs ≥ 2	0.99 (0.94-1.06)	0.87
Gentamicin	Yes	-	0.99 (0.96-1.01)	0.24
Oxytetracycline	No	≤ 0.5 vs ≥ 1	0.98 (0.95-1.0)	0.06
		≤1 vs ≥ 2	0.90 (0.87-0.92)	<0.01
		≤8 vs ≥ 16	0.90 (0.87-0.92)	<0.01

Penicillin	No	≤ 0.25 vs ≥ 0.5	1.05 (1.10-1.08)	<0.01
		≤ 0.5 vs ≥ 1	0.97 (0.95-1.0)	0.02
		≤1 vs ≥ 2	0.95 (0.91-0.99)	<0.01
		≤8 vs ≥ 16	0.95 (0.90-1.01)	0.09
Spectinomycin	Yes	-	0.83 (0.81-0.86)	<0.01
Tiamulin	Yes	-	0.85 (0.83-0.87)	<0.01
Tulathromycin	No	≤1 vs ≥ 2	0.73 (0.70-0.76)	<0.01
		≤ 2 vs ≥ 4	0.82 (0.78-0.85)	<0.01
		≤ 4 vs ≥ 8	0.89 (0.83-0.94)	<0.01
		≤ 32 vs ≥ 64	0.93 (0.86-1.01)	0.09
Sulphadimethoxine	-	≤ 256 vs > 256	0.82 (0.80-0.85)	<0.01
Trimethoprim-Sulphamethoxazole	-	≤ 2 vs > 2	1.02 (0.93-1.14)	0.61

Table S4-4. Changes in prevalence of antimicrobial resistance of *Haemophilus parasuis* isolates

Antimicrobial	Proportional odds assumption met	Reference MIC values vs higher MIC values (in μg/ml)	Time associated odds ratio	p-value
Ampicillin	Yes	-	1.20 (1.14-1.26)	<0.01
Ceftiofur	Yes	-	1.25 (1.17-1.33)	<0.01
Chlortetracyline	Yes	-	1.01 (0.94-1.08)	0.84
Enrofloxacin	Yes	-	1.34 (1.20-1.49)	<0.01
Florfenicol	Yes	-	1.09 (1.04-1.15)	<0.01
Gentamicin	Yes	-	1.14 (1.09-1.20)	<0.01
Oxytetracycline	No	≤ 0.5 vs ≥ 1	0.94 (0.89-1.0)	0.03
		≤1 vs ≥ 2	0.98 (0.92-1.03)	0.39

		≤ 4 vs ≥ 8	0.99 (0.92-1.07)	0.88
Penicillin	Yes	-	1.12 (1.07-1.18)	<0.01
Spectinomycin	Yes	-	1.03 (0.97-1.10)	0.3
Tiamulin	No	≤ 4 vs ≥ 8	0.94 (0.89-1.0)	0.05
		≤ 8 vs ≥ 16	1.02 (0.93-1.13)	0.62
Tulathromycin	Yes		1.23 (1.17-1.29)	<0.01
Sulphadimethoxine	-	≤ 256 vs > 256	0.88 (0.83-0.93)	<0.01
Trimethoprim-Sulphamethoxazole	-	≤ 2 vs > 2	1.05 (0.97-1.13)	0.22

CHAPTER-5 Genetic determinants of extended spectrum cephalosporin and fluoroquinolone resistance in clinical swine *E. coli* isolates

Introduction

Antimicrobial resistance has emerged as an issue of great concern in both human and veterinary medicine. Food animals are considered as potential reservoirs of antimicrobial resistant, zoonotic pathogens such as *Escherichia coli*, although the extent of spread of resistant bacteria via food chain is still debatable.[2] Antimicrobials that have critical importance in human medicine such as cephalosporins and fluoroquinolones are still used routinely in many parts of the world to treat diseased food animals, including swine in USA.[447,496] Furthermore, certain genetic mechanisms responsible for resistance to antimicrobials approved for use in animals (such as ceftiofur and enrofloxacin) and those used in human medicine (such as cefoxitin and ciprofloxacin) are the same.[497,498] It is therefore important to monitor the circulation of genes responsible for resistance to critically important/key antimicrobials, such as 3rd generation or higher cephalosporins and fluoroquinolones, in bacteria present in humans and animals to develop better source attribution models and targeted interventions in both humans and veterinary medicine.[499]

Resistance to extended-spectrum cephalosporins is mediated by extended spectrum beta-

lactamases (ESBLs), (commonly encoded by the *bla*$_{\text{TEM, SHV, CTX-M}}$ families of genes) and plasmidic AmpC (commonly encoded by the *bla*$_{\text{CMY}}$ family of genes).[343] These genes may be inserted on bacterial chromosomes but are usually present on plasmids which have the potential to disseminate horizontally to other bacterial strains. *bla*$_{\text{CTX-M}}$ genes are reported as the most prevalent ESBL encoders worldwide in humans and animals.[103]

However, ESBL-encoding genes were absent and *bla*$_{CMY}$ genes were primary responsible for extended spectrum cephalosporin resistance in bacteria of food animal origin in North America until the late 2000's.[500] However, recent reports have also suggested the emergence of ESBL genes in bacteria of food animal origin in USA over the last decade.[501]

Resistance to fluoroquinolones is mainly mediated by multiple chromosomal mutations in certain genes (*gyr*A, *gyr*B, *par*E and *par*C). Additionally, plasmid mediated quinolone resistance genes (such as *qnr*) and upregulation of efflux pumps confer variable levels of resistance to this antimicrobial family.[13] An increase in fluoroquinolone resistance was recently reported in *Salmonella* spp. isolates from diseased pigs in Minnesota between 2006 to 2015.[447] *qnr* genes encoded in plasmids were also found in *Salmonella* spp. isolates collected from retail pork, cecal samples from healthy pigs and clinical samples from diseased pigs in USA.[454,502,503]

Other antimicrobials which are critically important in human medicine include carbapenems, colistin and fosfomycin. Carbapenems are considered as last resort antimicrobials for severe infections in humans and are not used in food animals worldwide.[106] The genetic determinants for carbapenem resistance belong to *bla* family of genes that also confer resistance to cephalosporins and penicillins.[504] Colistin has been used in food animal medicine but has been recently reintroduced in human medicine as a "last resort" antimicrobial to treat multidrug resistant infections.[110] Colistin resistance has also gained considerable limelight in recent years due to the emergence of plasmid mediated mobile resistance genes (*mcr* genes) which have the potential to disseminate horizontally,[14] and the presence of *mcr* genes on plasmids also carrying ESBL-encoding

or carbapenem resistance genes.[505,506] Fosfomycin has been used for treating uncomplicated urinary tract infections in humans but its use is being considered in more complicated infections due to its activity against multidrug resistant pathogens.[507]

Although increasing information on the prevalence of phenotypic resistance in bacteria (including E. coli) of animal origin is generated thanks to national AMR monitoring programs, there is limited information on the genetic backbone mediating these resistance phenotypes. This may be of particular importance in the case of critically important antimicrobials such as fluoroquinolones, cephalosporins or carbapenems. The objective of this study was to characterize the genomic basis of fluoroquinolone and extended spectrum cephalosporin resistance in phenotypically resistant *E. coli* isolates collected from diseased pigs in USA between 2014-15 using short read whole genome sequencing (WGS). Both short and long read sequencing were used to characterize plasmids carrying ESBL, pAmpC and *qnr* genes which have not been previously reported in swine *E. coli* isolates in USA. The presence of *mcr-9* and fosfomycin resistance genes (*fos*A7) in swine *E. coli* isolates was also identified for the first time in USA.

Materials and methods

A total of 212 *E. coli* isolates from diseased pigs collected at the University of Minnesota Veterinary Diagnostic Laboratory (UMN-VDL) between 2014-2015 were included in this study. These isolates were selected on the basis of results of broth microdilution test routinely performed at the laboratory using CLSI guidelines (add citation to CLSI). Out of these 212 isolates, 93 had enrofloxacin MIC values ≥1 μg/ml and 106 had ceftiofur MIC values ≥8 μg/ml. Eighteen isolates had enrofloxacin MIC ≥1 μg/ml and ceftiofur

MIC ≥8 μg/ml and 45 isolates had MIC values <1 μg/ml for enrofloxacin and <8 μg/ml for ceftiofur.

These isolates were first subjected to short read sequencing using Illumina HiSeq 2500 (2 x 125bp). After initial quality control, draft genomes from these reads were assembled *de-novo* using Spades (version 3.13.0).[508] Draft genomes were uploaded to the Center for Genomic Epidemiology (CGE) webserver to identify multilocus sequence type (MLST),[509] acquired resistance genes (Resfinder),[510] plasmid sequence type (pMLST)[511] and plasmid replicon types (Plasmid Finder).[511] Chromosomal mutations in quinolone resistance determining regions (QRDRs) were identified by downloading sequences of *gyrA*, *gyrB*, *parC* and *parE* from reference *E. coli* k-12 genome (Genbank accession number-U00096) and performing nucleotide BLAST against the draft genomes locally (version 2.9.0, maximum hits-50, E-value threshold-10).

Core genomes of these 212 isolates were identified by first annotating draft genomes using PROKKA (version 1.13)[512] followed by core genome extraction using ROARY (version 3.11.2).[513] parSNPs program was used to identify high quality single nucleotide polymorphisms in these core genomes (version 1.0).[514] Maximum likelihood tree was built using general time-reversible with gamma substitution model using RaxML (version 8.0).[515] Support for nodes on trees was assessed using 5000 bootstrap replicates and visualization was done using iTOL (version 4.0).[516]

Additionally, long read sequencing was performed on a subset of isolates that were identified as carrying ESBL genes ($bla_{\text{SHV-12}}$, $bla_{\text{CTX-M}}$) in the analysis above using PacBio RSII technology (need to define the sequencer and chemistry used, and details about the data acquisition). Unicycler (version 0.4.7)[517] was used to perform *de-novo*

hybrid assemblies of these isolates using both long and short reads and assemblies were visualized using Bandage (version 0.8.1).[517] These complete genomes (here on referred to as "assembled plasmids") were uploaded to ISSaga webserver[518] for identification of insertion sequences and to the CGE webserver to perform analyses as mentioned above. These assembled plasmids were also blasted against a database of reference plasmids available at PLSDB webserver[519] and at NCBI blastn to identify previously described plasmids closely related to the plasmids assembled in this study. Plasmids with query coverage of >80% and nucleotide identity >90% were considered to be similar to assembled isolates in our study. BRIG (version 0.95)[520] was used to visualize the comparisons of identified closely related plasmids genomes with the assembled plasmids.

Putative plasmids were identified by mapping raw reads from short read sequences to the assembled plasmids and complete assemblies of plasmids carrying *bla*$_{\text{CMY-2}}$ and *qnr* genes published recently (Elnekave et al., 2019)[502] using BowTie2 (version 2.3.5.1).[521]

Results

Genetic mechanisms conferring fluoroquinolone and extended spectrum cephalosporin resistance

Out of 106 isolates with ceftiofur MIC values ≥8 μg/ml, 89 (84%) carried *bla*$_{\text{CMY-2}}$ genes (table 5-1). These genes were not present in rest of the 106 isolates with ceftiofur MIC values <8 μg/ml. The isolates carrying this gene belonged to 25 different ST types. ST12 (n=21) and ST101 (n=10) were the dominant ST types (clades 3 and 4, figure 5-1). Twenty of the ST12 isolates were potentially clonal and varied only by 9-32 single

nucleotide polymorphisms (SNPs). However, the isolates in ST10 and ST101 varied by 7-4185 and 8-1367 SNPs, respectively.

There were 24 isolates belonging to 13 different ST types which carried $bla_{\text{SHV-12}}$ and $bla_{\text{CTX-M}}$ genes, of which 21 were present in isolates with a ceftiofur MIC ≥8 μg/ml (table 5-1). The only *bla* gene found in isolates with ceftiofur MICs <8 μg/ml was $bla_{\text{SHV-12}}$.

The patterns of genetic determinants of fluoroquinolone resistance in 93 isolates with enrofloxacin MIC values ≥ 1 μg/ml is presented in table 5-2. These 93 isolates with enrofloxacin MIC values ≥1 μg/ml belonged to 27 different ST types. The dominant ST types were ST100 (n=37) and ST744 (n=17) (table 5-2). Thirty-six of these ST100 isolates varied by less than 20 SNPs, indicating potential clonality of these isolates (part of CC165, clade-1, figure 5-1). These ST100 isolates were collected from 6 different states in USA. In contrast, ST744 isolates varied by 8-606 SNPs (clade-2, figure 5-1).

Across all the 212 isolates under study, there were 6 different types of PMQR genes in 24 isolates spread across 7 states (table 5-3). Only 17 of these 24 isolates had enrofloxacin MIC ≥1 μg/ml (table 5-3). The 24 isolates belonged to 16 different ST types (table 5-3). Enrofloxacin MIC values for isolates with a single PMQR, two PMQRs and a PMQR with chromosomal mutation (*gyrA*- S83L, D87G or *parE*- D476A) ranged between 0.5-1.0 μg/ml, with the exception of two isolates that carried only *qnrB19* but had enrofloxacin MIC values of 2 μg/ml.

Description of assembled plasmids carrying PMQRs and ESBLs

We assembled complete plasmids using both long and short reads from eight isolates carrying $bla_{\text{CTX-M}}$ genes (table 5-4). These genes were present on IncFII and IncHI2

plasmids with size ranges of 69-240 kbp. *bla*$_{\text{CTX-M}}$ genes were present in regions flanked by IS*26*, IS*Ecp1*, IS*5*, IS*6* and Tn*3* family transposases, which were often truncated (table 5-4). In one isolate, *bla*$_{\text{CTX-M-15}}$ was present on the *E. coli* chromosome and the gene was flanked by transposases similar to those surrounding *bla*$_{\text{CTX-M-15}}$ in the IncF plasmids. Plasmids with *bla*$_{\text{CTX-M-14}}$ or *bla*$_{\text{CTX-M-27}}$ carried only these or one other AMR gene whereas the plasmids carrying *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{CTX-M-55}}$ genes carried at least two more AMR genes.

The two *bla*$_{\text{SHV-12}}$ genes were found on large assembled plasmids (approx. 300kbp), which were of IncHI2 type and carried genes for resistance to fluoroquinolones (*qnrB2*), aminoglycoside, sulphonamide, trimethoprim, tetracycline, penicillin and macrolide (Table 3). *bla*$_{\text{SHV-12}}$ genes were present in a region flanked by intact IS*6* transposases. One of these plasmids (p39SHV) also carried a *mcr-9* gene, which was present in a region surrounded by intact IS*5* and IS*6* transposases (table 5-4).

In addition to these ESBL-encoding plasmids, we identified and assembled a 59 kbp IncN plasmid carrying *qnrB77* gene using hybrid assemblies based on long and short reads (table 5-4). This plasmid was present in a ST4981 isolate which also carried an ESBL-encoding gene (*bla*$_{\text{CTX-M-15}}$) chromosomally. This plasmid also carried genes which confer resistance to trimethoprim, sulphonamides and aminoglycosides present on a class I integron. *qnrB77* gene was flanked by a complete and a truncated transposase of IS*91* family of insertion sequences (table 5-4).

On comparison of these assembled plasmids with the PLDSB database using nucleotide BLAST, we were able to identify previously described plasmids with a high similarity (>80% coverage, and >98% nucleotide identity). In brief, the plasmids carrying

ESBL encoding genes assembled in this study were similar to plasmids harbored on various *Enterobactericaceae* and collected from various sources (animals, humans, environment) across different continents and shared the same molecular context around genes of interest (*qnr*, *bla*). In contrast, we were not able to identify a previously described plasmid highly similar (>80% coverage) to $bla_{\text{CTX-M-15}}$ carrying IncFII (pMLST- F48:A1:B49) plasmids and *qnrB15* carrying IncN plasmid found in this study.

Description of putative plasmids carrying PMQRs and ESBLs

In 75 of the 89 $bla_{\text{CMY-2}}$-carrying isolates, putative plasmids alignments were obtained by mapping the raw reads to an IncA/C2 plasmid (accession number- MK191854.1; mean coverage- 74.3%, percent identity- >99%) and an IncI1 plasmid (Accession number- MK191846.1; mean coverage-92%, percent identity- >98%) (Table 5-5). These plasmids were recently described in *Salmonella enterica* isolates collected from diseased swine at UMN-VDL and reaffirm that these are the predominant plasmids disseminating extended spectrum cephalosporin resistance in bacteria of family Enterobacteriaceae in swine in USA.

By aligning raw reads with reference plasmids (published by Elnekave et al., 2019), putative plasmid alignments were identified for isolates carrying *qnrB19*, *qnrS2* and *qnrS1* (table 5-5). Putative plasmids carrying *qnrB19* and *qnrS2* ranged between 2757-3126 bp and were classified as ColRNAI-type plasmids encoding only a few proteins. Two isolates carrying *qnrS2* also aligned with IncQ type plasmids of nearly 7500bp length described recently by Elnekave et al. (2019). Putative plasmids carrying *qnrS1* genes were assembled by mapping raw reads to a reference plasmid (Accession number- MK356561.1). These putative plasmids were nearly 42kbp in size, belonged to

IncN family of plasmids, had a coverage of 72% and an identity of more than 99% as compared to the reference plasmid (table 5-5).

There were 36 isolates which carried either ESBL-encoding or *ampC* genes and PMQR or multiple mutations in QRDRs. There were four isolates which had a single mutation in QRDRs and also carried either ESBL or pAmpC genes. Five of the isolates carried a $bla_{\text{CMY-2}}$ gene and either $bla_{\text{CTX-M-27}}$, $bla_{\text{CTX-M-55}}$ or $bla_{\text{SHV-12}}$ gene.

Genetic mechanisms of other critical antimicrobials

No carbapenem resistance genes were present in these isolates. *mcr-9* gene was present in 6 isolates belonging to 5 different ST types. These isolates carried *mcr-9* gene and either an *ampC*, an ESBL or a PMQR gene. Descriptions of these isolates are presented in table 5-6. *mcr-9* was also present on one of the ESBL plasmids (p39SHV) assembled in this study (table 5-6).

Fosfomycin resistance gene (*fos*A7) was present in 7 of the isolates. Five of these isolates were of ST847 type and differed by only 11-57 SNPs. One of these isolates also carried *qnrS2* gene. Two isolates carrying *fosA7* belonged to ST75 and also carried $bla_{\text{CMY-2}}$ genes. Contigs carrying this gene were extracted and nucletotide BLASTn search was conducted. These contigs matched with chromosomal sequences of *E. coli* in 6 of these isolates with a query coverage and nucleotide identity of greater than 90%.

Discussion

Whole genome sequencing (WGS) of enrofloxacin and/or ceftiofur resistant *E. coli* revealed multiple mechanisms conferring resistance to these critical antimicrobials which were present on a wide spectrum of ST types recovered from the major swine producing states in USA. A combined approach using long and short read WGS technologies

reaffirmed the widespread presence of certain genetic determinants that could contribute to the spread of AMR, such as plasmids carrying *bla*$_{CMY-2}$, which have established in *Salmonella* and *E. coli* isolates circulating in food animal production in the past.[500] We also assembled plasmids which have not been described previously in swine or other food animals and retail meat in USA such as those carrying ESBL encoding genes and compared them to previously described plasmids which were isolated in other countries, indicating that these plasmids carrying ESBL encoding genes were part of a pandemic spread of these plasmids.

Nearly 84% of the ceftiofur resistant isolates (MIC ≥8 μg/ml) carried a *bla*$_{CMY-2}$ gene, which is consistent with findings in ceftiofur-resistant *Salmonella* isolates from diseased pigs collected during same study period.[502] However, 24 *E. coli* isolates in this study (including 3 with ceftiofur MICs<8 μg/ml) carried a *bla*$_{CTX-M}$ or *bla*$_{SHV-12}$ gene. In comparison to ceftiofur resistant *Salmonella* spp. isolated from diseased pigs in USA,[502] we reported a much higher prevalence (18%) of *bla*$_{CTX-M}$ in our isolates. Nevertheless, according to our findings the distribution of *bla*$_{CTX-M}$ genes is more limited compared with reports in ESBL- *E. coli* isolates retrieved from swine in European and Asian countries (range-33 to 100%) (Chapter 2). ESBL-encoding genes are the predominant genes responsible for extended spectrum cephalosporin resistance globally in food animals (Bevan et al., 2016). However, until the late 2000's these genes were not found in food animal isolates collected in North America.[522] In a study on *E. coli* isolates collected from diseased pigs at UMN-VDL in 2008, all ceftiofur resistant isolates carried *bla*$_{CMY-2}$ genes[58]; whereas *bla*$_{CTX-M}$ carrying *E. coli* in finishing pigs in USA were first identified in 2011.[367] There are a few more recent studies that have reported the sporadic

occurrence of *bla*$_{\text{CTX-M}}$ genes in *Enterobacteriaceae* isolates of swine origin (including pork) in USA.[523,524]

Similar to ESBLs, presence of PMQRs (*qnr, aac*) in food animal isolates in USA has not been reported until recently.[450,454,502,525] PMQR genes identified in this study such as *qnrB* and *qnrS* have also been reported from *E. coli* and *Salmonella* isolates of swine origin in Asia, Australia and Europe. There has also been an increase in PMQR genes is *Salmonella* isolates collected from diseased humans in USA and animal sources might contribute to this surge.[525] The presence of PMQR genes without presence of chromosomal mutations in QRDR was able to elevate MIC values to intermediate levels (1 μg/ml) but not to higher MIC levels. This is consistent with the previous reports that PMQRs like *qnrB* and *qnrS* confers lower level resistance to quinolones by inhibiting binding of quinolones to DNA gyrase.[526] However, these PMQRs are known to supplement resistance caused by other mechanisms such as altered target enzymes (DNA gyrase), efflux pump activities and deficiencies in outer membrane porin channels.[527] The presence of PMQRs in zoonotic bacteria and their clinical impact on both human and animal health should be continuously monitored.

The presence of a large number of different ST types along with a few isolated clones of *E. coli* carrying PMQRs, ESBLs and chromosomal mutations in QRDRs across major swine producing states in USA is indicative of widespread horizontal and clonal dissemination of these genetic mechanisms. This pattern of both clonal and horizontal transmission is consistent with recent studies in Germany where clones of certain ST types (ST167 and ST410) of *E. coli* were associated with carriage of *bla*$_{\text{CTX-M-15}}$ while overall there were 26 ST types carrying this gene.[528,529]

ESBLs have been associated with pandemic ST131 *E. coli* in humans.[530] However, in this study only one ST131 isolate was identified, and it was considered susceptible to both antimicrobial classes under study. The main swine specific ST type with enrofloxacin MIC (≥1 μg/ml) identified in this study was ST100, which is associated with porcine enterotoxigenic infections.[128] Enrofloxacin has been introduced to treat swine enteric infections in USA since 2012[447] and the association of ST100 with enrofloxacin resistance might be of concern to swine health. Some of the major cephalosporin and /or fluoroquinolone resistant ST types (ST744, ST10CC, ST86CC, ST23CC, ST58) identified in our panel have been associated with carriage of $bla_{\text{CTX-M}}$ in multiple animal species, have been implicated in human infections and are considered "zoonotic ST types".[531–535] The presence of these resistant ST types in swine may suggest a potential risk of spread to other animal species including humans.

Putative plasmids carrying $bla_{\text{CMY-2}}$, *qnrS1, qnrS2, qnrB2* and *qnrB19* were aligned using complete plasmid assemblies described by Elnekave et al. (2019)[502] and we refer the readers to this article for further details on the above-mentioned plasmids. To the best of my knowledge, this is the first study to describe completely assembled plasmids carrying $bla_{\text{CTX-M-14, -15, -27, -55}}$, $bla_{\text{SHV-12}}$ and *qnrB15* in *E. coli* isolates of swine origin in USA. However, the close identities between some plasmids in this study and those already described in humans and animals globally indicate that the presence of ESBL genes in this isolate collection could be a part of the pandemic expansion of ESBLs.[103] $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-14}}$ are considered to be predominant ESBL genes in humans globally[103] and have also been identified in food animals including pigs worldwide (Chapter-2). The plasmid carrying $bla_{\text{CTX-M-15}}$ (p1CTX) in our study was

highly similar (98% coverage, >99% nucleotide identity) to the ones identified in human *E. coli* isolates collected in USA between 2009-10,[536] (Genbank accession number- CP009232) which were further described to have the same plasmid backbone as ESBL gene carrying plasmids worldwide.[536] *bla*$_{\text{CTX-M-27}}$ carrying plasmids identical to those in this study have been previously isolated from humans in USA[537] and England[538] and sick ducks in China[539]. *bla*$_{\text{CTX-M-55}}$ carrying plasmids similar to ones in this study have been isolated from various human and animal sources in South Korea, China and England (unpublished, Genbank accession numbers- KM396298.1, KM396299.1, KM396300.1, NZ_CP0309401.1, NZ_CP036178.1, MG014721.1, MK169211.1). *bla*$_{\text{CTX-M-14}}$ carrying plasmids identical to those in this study have been previously isolated from humans in Hong Kong and has been characterized as an epidemic plasmid type (pHK01)[540] which has spread globally to other Asian countries (China, Vietnam, South Korea) and European countries (Finland) (unpublished, Genbank accession numbers- NC_013727.1, KU932024.1, KU987452.1, NC_013542.1, NZ_CP018973.1). *bla*$_{\text{SHV-12}}$ carrying plasmids similar to ones in this study have been isolated from various human and animal sources in USA,[541] China[542], Taiwan[543] and Denmark[544]. Moreover, these plasmids were isolated from different bacterial species such as *Salmonella*, *Klebsiella*, *Enterobacter*, *E. coli*–ST131 etc. and had identical genetic contexts around *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{SHV-12}}$ genes as in plasmids assembled in this study. Insertion sequences (IS*26*, IS*Ecp9*, IS*6*) that were part of the above-mentioned genetic contexts have been demonstrated to be successful in transposing ESBL-encoding genes across plasmids and bacterial chromosomes.[545]

It has been widely believed that the presence of plasmids in the absence of selective pressure imposes a metabolic fitness cost to the bacterial host.[546] However, the

fitness cost imposed due to plasmid carriage depends on the plasmid-bacterial host combination.[547–549] There are several plasmid characteristics which help in plasmid stability in bacterial hosts. For example, IncF plasmids have a narrow host range and carry factors such as addiction systems, post-segregational killing machinery etc. which help in maintaining their stability in bacterial hosts without antimicrobial pressure.[550] Similarly, IncHI2 plasmids carry genes which confer resistance to heavy metals, mutagenesis induction system etc. which aid in their stability.[551] Endemic plasmids identical to those found in our study such as pHK01-like plasmids have been demonstrated to be conjugative *in-vitro*.[552] Hence, it can be postulated that these plasmids might aid in establishment of ESBLs as dominant mechanisms behind extended spectrum cephalosporin resistance in swine in USA as has happened globally.

This is the first report of the presence of *mcr-9* and *fosA7* in any bacteria from food animals in USA. *mcr-9* gene was recently described for the first time in a *S. Typhimurium* isolate collected from a human patient in Washington State, USA and was able to confer colistin resistance to *E. coli* isolates cloned with this gene.[553] It should be noted that colistin has never been used in swine production in USA and the presence of *mcr-9* gene in the absence of colistin use could be an indicator of the complex transmission dynamics of resistant mechanisms across different ecosystems and/or co-selection of resistant mechanisms due to use of unrelated antimicrobials. We also reported the presence of *fosA7* gene in *E. coli* isolates of swine origin in USA. *fosA7* gene was discovered only recently in *S.* Heidelberg isolates from poultry in Canada[554] but never been described in *E. coli*. Moreover, fosfomycin resistance genes are thought to be plasmid mediated in *E. coli* isolates[555] but the contigs carrying these genes in this study

matched with chromosomal sequences available in Genbank database. Further analyses of isolates carrying *mcr-9* and *fosA7* is needed

Several limitations must be considered when interpreting these results. These isolates were collected from diseased pigs submitted for diagnostic purposes that might therefore have been treated with antimicrobials before submission. An association between antimicrobial use and presence of these genes should not be established on the basis of these results until further studies are conducted. Also, diseased pigs are removed from the food chain and therefore the presence of resistant and potentially zoonotic ST types does not imply an immediate risk for public health. Nevertheless, the putative plasmids carrying *qnr* genes found here were identical to those isolated from *Salmonella* spp. collected from retail pork and cecal samples from healthy pigs,[454] suggesting they may eventually go through the food processing chain. Hence, these isolates might still indicate a potential reservoir for human infections and WGS data presented here can aid in making better source attribution models in the future.

Conclusions

We were able to identify and describe novel genetic mechanisms of resistance to some critical antimicrobial classes important to both human and animal health in swine clinical *E. coli* isolates, some of which had never been described in isolates of animal origin in the US. Future studies will focus on assembling finished genomes of isolates carrying *mcr-9* and *fosA7* genes as well as conducting conjugation and fitness experiments on selected isolates to predict the success of these plasmids and bacterial hosts.

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Tables
Table 5-1. Number and type of ESBL and pAmpC genes and associated ST types in 106 ceftiofur-resistant ((MIC ≥ 8 μg/ml) *E. coli* clinical isolates of swine origin

Type of gene	Number of Resistant isolates (% of isolates harboring the gene)	ST types (n= number of isolates)	Collection location
bla$_{\text{CMY-2}}$	89 (84.0%)	12 (n=21), 10 (n=12), 101 (n=10), 100 (n=9), 58 (n=6), 23 (n=4), 410 (n=4), 90 (n=3), 744 (n=3), 75 (n=2), 69 (n=1), 73 (n=1), 88 (n=1), 93 (n=1), 127 (n=1), 154 (n=1), 224 (n=1), 369 (n=1), 761 (n=1), 977 (n=1), 2025 (n-1), 3057 (n=1), 4373 (n=1), 6234 (n=1)	MN, CO, IA, IL, KS, MI, MO, NE, OH, OK, SD, TN, TX, WI
bla$_{\text{CTX-M-15}}$	9 (8.5%)	617 (n=2), 744 (n=2), 4981 (n=2), 167 (n=1), 410 (n=1), 58 (n=1)	MN, IL, OK

bla$_{CTX-M-55}$	5 (4.7%)	457 (n=2), 101 (n=1), 165 (n=1), 744 (n=1)	MN, MO, KS
bla$_{CTX-M-14}$	2 (1.9%)	10 (n=2)	IL
bla$_{SHV-12}$*	2 (1.9%)	10 (n=2), 641 (n=2), 1112 (n=1)*	MN, NE, IL, OK
bla$_{CTX-M-27}$	3 (2.8%)	10 (n=1), 744 (n=1), 1585 (n=1)	MN, NE, OK

*- 3 of these isolates had ceftiofur MIC value <8 μg/ml

Table 5-2. Pattern of genetic determinants of fluoroquinolone resistance in *E. coli* clinical isolates of swine origin with enrofloxacin MIC (≥1 μg/ml)

MIC value (in μg/ml)	Pattern of genetic determinants (n=number of isolates)	ST types (n=number of isolates)
>2	*gyrA*(S83L) + *gyrA*(D87Y or D87N or D87G) + *parC*(S80I or S80R) ± *parC*(A56T or E84G) ± *parE*(S458A or L416F) ± single PMQR (*aac(6')-Ib-cr, qnrB77, qnrB15 or qnrB19*) (n=49)	744 (n=11), 100 (n=10), 224 (n=4), 410 (n=3), 10 (n=2), 457 (n=2), 617 (n=2), 4981 (n=2), 88 (n=1), 93 (n=1), 167 (n=1), 977 (n=1), 1585 (n=1), 2161 (n=1), 3901 (n=1)

2	*gyrA*(S83L) + *parC*(S80I or S80R) (n=23)	100 (n=21), 58 (n=1), 90 (n=1)
	qnrB19 (n=2)	361 (n=1), 2496 (n=1)
	No genetic determinants (n=1)	5926 (n=1)
1	*gyrA*(S83L) + *parC*(S80I or S80R) (n=7)	100 (n=6), 69 (n=1)
	gyrA(S83L) only (n=1)	6234 (n=1)
	gyrA(D87G) + *qnrB2* (n=1)	10 (n=1)
	qnrB19 + *qnrS2* (n=1)	101 (n=1)
	aac(6')-Ib-cr + *qnrB2* (n=1)	540 (n=1)
	Single PMQR (*qnrB19*, *qnrS1*, *qnrS2*, *qnrB2* or *qnrB15*) (n=6)	10 (n=3), 641 (n=1), 847 (n=1), 5759 (n=1)
	No genetic determinants (n=1)	10 (n=1)

Table 5-3. Number and type of PMQRs and associated ST types in *E. coli* of swine origin

Type of gene (n=number of isolates)	ST types	Collection location	Number of isolates with enrofloxacin MIC (<1 µg/ml) (n=119) carrying PMQR genes	Number of isolates with enrofloxacin MIC (=>1 µg/ml) (n=93) carrying PMQR genes
qnrB2 (n=6)	10, 540, 641, 1112	MN, IL, IA	3	3
qnrB19 (n=6)	93, 101, 361, 2161, 2496, 5759	TX, IA, KS, OK	1	5
qnrB15 (n=3)	10, 100, 4981	MN, IA, OK	0	3
qnrS1 (n=2)	10, 641	MN, KS	0	2
qnrS2 (n=4)	10, 101, 847	MO, OK	2	2
aac(6')-*lb-cr* (n=6)	58, 410, 540, 617, 641	MN, IL	2	4

Table 5-4. Characteristics of plasmids assembled in this study

Isolate no.	Gene of interest	Size of plasmid	Replicon typing (pMLST)	ST type	Other AMR genes	Mobile genetic elements flanking *bla* and *qnr* genes
p1CTX	*bla*$_{\text{CTX-M-15}}$	170 kbp	IncF (F31:A4:B1)	617	*aadA5, aac(3)-IIa, aac(6')-Ib-cr, bla*$_{\text{OXA-1}}$*, mph(A), catB3, sul1, tet(B), dfrA17*	ΔIS*Ecp1- bla*$_{\text{CTX-M-15}}$-Tn*3*
p2CTX	*bla*$_{\text{CTX-M-15}}$	170 kbp	IncF (F31:A4:B1)	58	*aac(6')-Ib-cr, bla*$_{\text{OXA-1}}$*, mph(A), tet(B), dfrA17*	ΔIS*Ecp1- bla*$_{\text{CTX-M-15}}$-Tn*3*
p4CTX	*bla*$_{\text{CTX-M-15}}$	115 kbp	IncF (F48:A1:B49)	744	*aac(3)-IIa, bla*$_{\text{TEM-1b}}$*, mph(A), dfrA17*	IS*Ecp1- bla*$_{\text{CTX-M-15}}$-IS*26*
23	*bla*$_{\text{CTX-M-15}}$	Chromosomal		4981	*mdf(A)*	ΔIS*Ecp1- bla*$_{\text{CTX-M-15}}$-Tn*3*
p23qnr	*qnrB15*	60 kbp	IncN (unknown)	4981	*aac(3)-Iid, aadA2, aph(3')-Ia, aph(6)-Id, aph(3'')-Ib, bla*$_{\text{TEM-1b}}$*, mph(A), sul1, tet(M), dfrA12*	IS*91-qnrB15*-IS*91*

p33SHV	*bla*$_{SHV-12}$	300 kbp	IncHI2 (ST-1)	641	*aac(6')-Ib3, aac(6')-Iic, aph(6')-Id, aph(3'')-Ib, aadA2,* *bla*$_{TEM-1b}$*, aac(6')-Ib-cr, qnrB2, ere(A), sul1, sul2, dfrA19*	IS*26*- *bla*$_{SHV-12}$-IS*26*
p37CTX	*bla*$_{CTX-M-27}$	69 kbp	IncF (F21*:A-:B-)	744	*erm(B)*	ΔIS*Ecp1*- *bla*$_{CTX-M-14}$-IS*5*
p39SHV	*bla*$_{SHV-12}$	300 kbp	IncHI2 (ST-1)	1112	*aph(3'')-Ib, a[h(6)-Id, aph(3')-Ia, aac(6')-IIc,* *bla*$_{TEM-1b}$*, mcr-9, ere(A), catA2, sul1, tet(D)*	IS*26*- *bla*$_{SHV-12}$-IS*26*
p65CTX	*bla*$_{CTX-M-55}$	240 kbp	IncHI2 (ST-2)	165	*aac(3)-IIa,* *bla*$_{TEM-1b}$*, mph(A), dfrA17*	ΔIS*Ecp1*- *bla*$_{CTX-M-55}$-ΔIS*6*
p62CTX	*bla*$_{CTX-M-27}$	69 kbp	IncF (F21*:A-:B-)	10	-	IS*5*- *bla*$_{CTX-M-27}$-ΔIS*Ecp1*
p77CTX	*bla*$_{CTX-M-14}$	77 kbp	IncF (F2:A8:B56)	10	-	3'-5'exonuclease- *bla*$_{CTX-M-14}$-IS*Ecp1*

Table 5-5. Characteristics of putative plasmids aligned with reference plasmids in this study

Gene	Accession number of reference plasmid	Size of reference plasmid	Replicon type of reference plasmid (pMLST)	Number of putative plasmids aligned with this reference plasmid	Average size of putative plasmid in Kbp (range of size)	Average percent nucleotide identity of putative plasmid
bla$_{CMY-2}$	MK191845.1	179 kbp	IncA/C2 (ST-3)	54	133.4 (91.3-177.2)	>99%
bla$_{CMY-2}$	MK191846.1	99 kbp	IncI1 (ST-12)	21	91.3 (85.1-98.0)	>98%
bla$_{CTX-M-14}$	p77-CTX-M	77 kbp	IncF (F2:A8:B56)	1	74	>99%
bla$_{CTX-M-15}$	p1-CTX-M	171 kbp	IncF (F31:A4:B1)	3	132 (101-161)	>99%
bla$_{CTX-M-15}$	p4-CTX-M	113 kbp	IncF (F48:A1:B49)	2	92 (71-113)	>99%
bla$_{CTX-M-27}$	p37-CTX-M	75 kbp	IncF (F21*:A-:B-)	3	69 (59-74)	>99%
bla$_{CTX-M-55}$	p65-CTX-M	240 kbp	IncHI2 (ST-2)	1	201	>99%
bla$_{SHV-12}$	p33-SHV	301 kbp	IncHI2 (ST-1)	3	289 (281-293)	>99%
qnrB15	p23-qnr	59 kbp	IncN	2	57 (57-57)	>99%
qnrB19	MK191839.1	3.1 kbp	ColRNAI	2	3.1 (3.1-3.1)	>99%

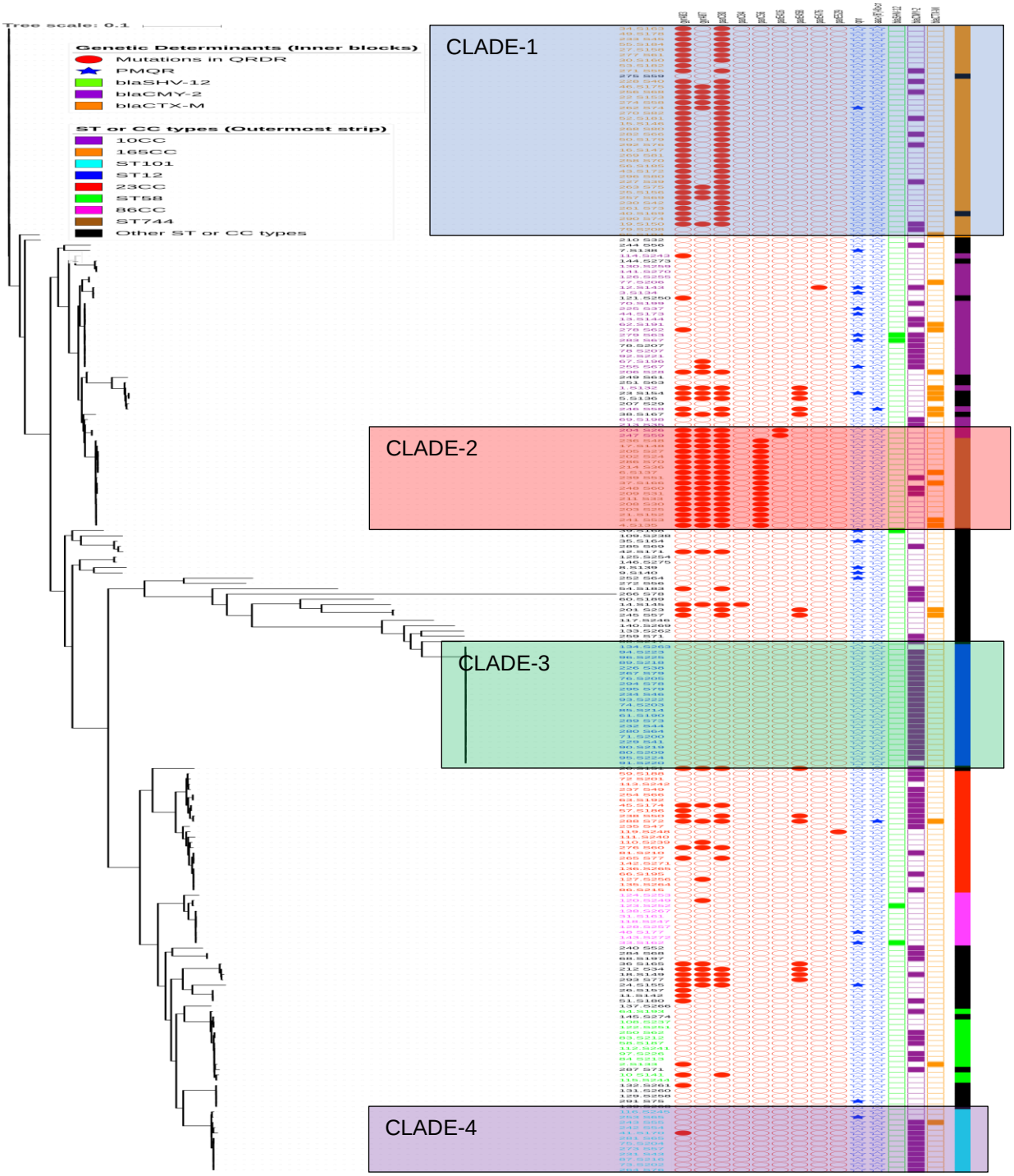
qnrS1	MK356561.1	59 kbp	IncN	2	43 (42-43)	>99%
qnrS2	CP032896.1	7.6 kbp	IncQ2	2	7.6 (7.6-7.6)	>99%

Table 5-6. Characteristics of isolates carrying *mcr-9* genes

Isolate	ST type	Other AMR genes in the isolate
8	540	*qnrB2,bla*$_{\text{TEM-1b}}$*,aac(6')-IIc,aadA2,strA,strB, sul1,sul2,sul3,ere(A)-like,dfrA12,dfrA18-like,aacA4,aac(6')Ib-cr-like*
81	90	*floR-like,bla*$_{\text{CMY-2}}$*,bla*$_{\text{TEM-1b}}$*,aac(3)-Via-like,aadA2,aadA5,aph(3')-Ia-like,armA,strA,strB,sul2,mph(E),msr(E),dfrA1*
243	101	*catA1-like,bla*$_{\text{CTX-M-15}}$*;blaTEM-1B,aph(3')-Ia-like;aac(3)-Iia;aadA1-like;aph(6)-Id;aph(3'')-Ib,sul2,ph(A),tet(B)-like*
279	10	*qnrB2,bla*$_{\text{SHV-12}}$*;bla*$_{\text{TEM-1b}}$*;bla*$_{\text{CMY-2}}$*,strA;aph(3')-Ia-like;aac(6')-Iic;aadA2-like;aph(6)-Id,sul1;sul2,ere(A)-like,tet(D);tet(B)*
283	10	*qnrB2,bla*$_{\text{SHV-12}}$*;bla*$_{\text{TEM-1b}}$*;bla*$_{\text{CMY-2}}$*,aph(6)-Id;aph(3')-Ia-like;aac(6')-Iic;aadA2-like;strA,sul1;sul2,ere(A)-like,tet(D);tet(B)*

Figure

Figure 5-1. Maximum likelihood tree representing the phylogenetic relationships between different isolates included in this study



CHAPTER-6 General Discussion

This dissertation provides new data that can improve our understanding on the global situation of resistance to critical antimicrobials in indicator bacteria from swine, on the prevalence of AMR in *E. coli* and other bacterial pathogens (*S. suis, P. multocida, A. suis* and *H. parasuis*) isolated from diseased pigs in USA, and on the genomic determinants conferring extended spectrum cephalosporin and fluoroquinolone resistance in selected swine clinical *E. coli* isolates collected in USA. In chapter 2, we conducted a systematic review and meta-analysis to estimate the global prevalence of cephalosporin, quinolone, colistin and carbapenem resistance in *E. coli* from different swine sources. Although it is widely reported that human isolates collected in LMIC are more resistant to different antimicrobials compared to isolates from UIC, such comparisons between AMR in UIC and LMIC isolates from food animals have not been performed on a global scale. In our study we also observed higher prevalences of AMR in swine *E. coli* isolates from LMIC compared to UIC across different sources (healthy pigs, diseased pigs, porcine meat products). It was encouraging to see that the prevalence of carbapenem resistance was globally very low in swine *E. coli* isolates with a few exceptions. Carbapenems are the last class of antimicrobials introduced in human medicine and are effective against a broad spectrum of bacteria. Ideally these low levels should be maintained and constantly monitored in swine *E. coli* isolates due to their importance from a public health point of view. Another interesting finding was that data availability and consistency was higher in UIC compared to LMIC. For some of the bacteria-antimicrobial combinations in LMIC, data available was coming from only a few studies or even a single study, leading to huge uncertainty in the estimates. Simply put, we have an indication of moderate to high

prevalence of resistance to critical antimicrobials but the lack of reliable information hinders much of the prevalence and trend analyses conducted. It would be advisable for countries such as India, China and Vietnam to include swine populations in their AMR surveillance activities as they set up their programs. Similarly, the number of studies focusing on prevalence of genetic determinants conferring resistance, as well as the number of isolates in these studies, were lower for articles from LMIC compared to UIC except for China. Hence, genotypic prevalence of AMR in isolates from LMIC remains understudied and future studies should incorporate components of genomic surveillance, particularly in LMIC.

In chapter 3, changes in prevalence of single and multiple AMR phenotypes in swine clinical *E. coli* isolates in USA were studied. The patterns of infdvidual AMR remained realtively stable or changed modestly for all of the antimicrobials evaluated except enrofloxacin, for which the prevalence of AMR increased drastically from 2008 onwards. Enrofloxacin has only been recently introduced for treatment of swine enteric and respiratory diseases in USA, and based on our findings the prevalence of enrofloxacin resistance in isolates coming from various sources (healthy pigs, diseased pigs, pork) should be continuously monitored, and use of enrofloxacin be regulated as needed. Changes in multiple AMR was estimated by classifying an isolate as being multiple antimicrobial resistant if they are resistant to 3 or more than antimicrobial classes and running logistic regression model. This model revealed a slight decrease in the proportion of isolates carrying multiple AMR over the study-period. However, resistotype analysis using rarefaction curves and networks of AMRs revealed an increase in the number of AMR combinations and increased strengths and density of AMR

networks over the years. This increase in AMR complicates the threat posed by resistant bacteria as use of antimicrobials can co-select for different classes of antimicrobials. These AMR networks should be treated as hypothesis generating tools, and field trials can further elucidate how the use of different antimicrobials can co-select for other antimicrobial classes. For example, associations between ceftiofur usage on resistance to other antimicrobials (e.g. florfenicol) can be estimated in actual field conditions to check whether the correlation between ceftiofur-florfenicol resistance observed in this chapter holds true in field conditions. Changes in AMR should be surveyed continously using diagnostic data starting January 2017 and compared with the results presented here. This comparison will likely provide an indication of success or failure of veterinary feed directive in reducing prevalence of AMR. Additionally, antimicrobial usage data should be incorporated in these univariate nad multivariate models to elucidate the extent of antimicrobial usage on evolution of AMR.

In chapter 4, we assessed the presence of changes in the prevalence of AMR in certain swine bacterial pathogens (*S. suis, P. multocida, A. suis* and *H. parasuis*) isolated in USA. The prevalence of AMR for those bacteria-antimicrobial combination for which clinical breakpoints were available remained consistently low (< 12%) with the only exception of tetracyclines. This suggest that certain antimicrobials have remained clinically effective against *S. suis* and *P. multocida.* However, for bacteria-antimicrobial combinations with no breakpoints available, ordinal regression models estimated significant increases or decreases in the odds of having higher MIC values. Lack of availability of clinical breakpoints or epidemiological cut-offs significantly hampered the interpretation of results coming fromthese regression models though, as we were not able

to make strong clinical or epidemiological inference based on them and secondly, the results were contradictory for several bacteria-antimicrobial combinations despite using the least parsimonious ordinal regression models with odds decreasing at certain MIC level but increasing for some other MIC levels for same antimicrobial-bacteria combination. Statistical models (multivariate network of AMR in chapter 3, ordinal regression models in chapter 4) based on the analysis of MIC values without applying breakpoints can provide an indication of an increase or decrease in AMR above/below these, but the clinical decision making and interpretation and reporting of results to stakeholders can become more difficult. A combined statistical approach where breakpoints can be used to make binary logistic regression models (as done in chapters 1 and 2) and ordinal regression models based on MIC distributions (chapter 4) that can detect minor shifts in MIC values can serve as a complete and interpretable alternative for the analysis of AMR. Authors have also used breakpoints from related bacteria as a proxy to study the prevalence of AMR. Our study suggests that this is a rather limited approach to circumvent the absence of breakpoints and should be avoided. Future studies should focus instead on establishing both phenotypic and/or genotypic breakpoints for bacteria critical for swine health such as *H. parasuis* and *A. suis*.

In chapter 5, the presence of genetic determinants of extended-spectrum cephalosporins and fluoroquinolone resistance was assessed in selected swine *E. coli* isolates presenting different resistance phenotypes. We found a higher proportion of ceftiofur resistant *E. coli* carrying ESBL encoding genes compared to previous studies. These ESBL encoding genes have become the dominant mechanism behind resistance to 3rd or higher generation cephalosporins and their prevalence should be continuously

monitored to establish whether these genes become dominant in swine bacterial populations in the near future as has happened in other parts of the world. Indeed, plasmids carrying these genes were similar to plasmids that have been involved in the global expansion of ESBL encoding genes, and these plasmids have been successful in the dissemination of extended spectrum cephalosporin resistance both *in-vitro* and *in-vivo*. Plasmid mediated resistance genes and chromosomal mutations alone or in combinations were responsible for conferring various degrees of enrofloxacin resistance in the assessed isolate collection. PMQR genes and plasmids similar to those previously reported in isolates from retail pork and healthy pigs were found in our collection, which gives strength to the hypothesis that studying AMR in clinical isolates can be indicative of emerging resistance determinants in healthy animal populations and in turn, in the food chain. The complete plasmid assemblies generated in this study can be used for genomics-based source attribution models in the future. Future studies should include measuring the fitness of isolates carrying ESBL encoding genes and their potential for dissemination in *in-vitro* and *in-vivo* conditions. The plasmids and *E. coli* genomes drafted in this study can also be compared with those drafted from humans or other food animals retrospectively and prospectively to strengthen source-attribution models that are based on genetic data.

In conclusion, these studies provide an insight into the changes in phenotypic and genotypic prevalence of AMR in different bacteria of animal and human health importance collected from clinically affected swine populations in USA. Additionally, this thesis also includes a comprehensive systematic review of the global prevalence of resistance to critical antimicrobials in isolates recovered from different swine sources,

which helps putting the results on AMR distribution in USA isolates in a global context and also highlights the need to include and improve surveillance of AMR in swine populations in LMIC.

References

1. O'Neill J. *Tackling Drug-Resistant Infections Globally: Final Report and Recommendations*. HM Government and Welcome Trust: UK https://amr-review.org/sites/default/files/160525_Final%20paper_with%20cover.pdf. Accessed August 1, 2019.

2. Aarestrup FM. The livestock reservoir for antimicrobial resistance: A personal view on changing patterns of risks, effects of interventions and the way forward. *Philos Trans R Soc B Biol Sci*. 2015;370(1670):20140085. doi:10.1098/rstb.2014.0085

3. Barton MD. Impact of antibiotic use in the swine industry. *Curr Opin Microbiol*. 2014;19(1):9–15. doi:10.1016/j.mib.2014.05.017

4. Mellon M, Benbrook C, Benbrook KL. Estimates of Antimicrobial Abuse in Livestock. :14.

5. Zurfluh K. Quinolone resistance mechanisms among extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli i*solated from farm animals in Switzerland. *Schweiz Arch Für Tierheilkd*. 2015;157(1):59-62. doi:10.17236/sat00006

6. FDA. Food and Drug Administration CFR Parts 514 and 558. https://www.govinfo.gov/content/pkg/FR-2015-06-03/pdf/2015-13393.pdf.

7. Levy S. Reduced Antibiotic Use in Livestock: How Denmark Tackled Resistance. *Environ Health Perspect*. 2014;122(6). doi:10.1289/ehp.122-A160

8. Glass-Kaastra SK, Pearl DL, Reid-Smith R, et al. Multiple-class antimicrobial resistance surveillance in swine *Escherichia coli* F4, *Pasteurella multocida* and *Streptococcus suis* isolates from Ontario and the impact of the 2004–2006 Porcine Circovirus type-2 Associated Disease outbreak. *Prev Vet Med*. 2014;113(2):159-164. doi:10.1016/j.prevetmed.2013.11.008

9. Mather AE, Reeve R, Mellor DJ, et al. Detection of rare antimicrobial resistance profiles by active and passive surveillance approaches. *PLoS ONE*. 2016;11(7):1–12. doi:10.1371/journal.pone.0158515

10. Singer RS, Williams-Nguyen J. Human health impacts of antibiotic use in agriculture: A push for improved causal inference. *Curr Opin Microbiol*. 2014;19:1-8. doi:10.1016/j.mib.2014.05.014

11. Gibbons JF, Boland F, Egan J, et al. Antimicrobial Resistance of Faecal *Escherichia coli* Isolates from Pig Farms with Different Durations of In-feed Antimicrobial Use. *Zoonoses Public Health*. 2016;63(3):241–250. doi:10.1111/zph.12225

12. Love WJ, Zawack KA, Booth JG, Gröhn YT, Lanzas C. Markov Networks of Collateral Resistance: National Antimicrobial Resistance Monitoring System Surveillance Results from *Escherichia coli* Isolates, 2004-2012. *PLOS Comput Biol*. 2016;12(11):e1005160. doi:10.1371/journal.pcbi.1005160

13. Holmes AH, Moore LSP, Sundsfjord A, et al. Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*. 2016;387(10014):176-187. doi:10.1016/S0140-6736(15)00473-0

14. Liu YY, Wang Y, Walsh TR, et al. Emergence of plasmid-mediated colistin resistance mechanism *mcr-1* in animals and human beings in China: A microbiological and molecular biological study. *Lancet Infect Dis*. 2016;16(2):161–168. doi:10.1016/S1473-3099(15)00424-7

15. Machuca J, Briales A, Labrador G, et al. Interplay between plasmid-mediated and chromosomal-mediated fluoroquinolone resistance and bacterial fitness in *Escherichia coli*. *J Antimicrob Chemother*. 2014;69(12):3203-3215. doi:10.1093/jac/dku308

16. United States Department of Agriculture. *Census of Agriculture, United States, Summary and State Data*.; 2019. https://www.nass.usda.gov/Publications/AgCensus/2017/Full_Report/Volume_1,_Chapter_1_US/usv1.pdf. Accessed August 1, 2019.

17. Etienne J, Chirico S, Gunabalasingham T, Dautzenberg S, Gysen S. EU Insights–Perceptions on the human health impact of antimicrobial resistance (AMR) and antibiotics use in animals across the EU. *EFSA Support Publ*. 2017;14(3):1183E.

18. Giubilini A, Birkl P, Douglas T, Savulescu J, Maslen H. Taxing Meat: Taking Responsibility for One's Contribution to Antibiotic Resistance. *J Agric Environ Ethics*. 2017;30(2):179-198. doi:10.1007/s10806-017-9660-0

19. Blyth DM, Mende K, Maranich AM, et al. Antimicrobial resistance acquisition after international travel in U.S. travelers. *Trop Dis Travel Med Vaccines*. 2016;2(1):4. doi:10.1186/s40794-016-0020-2

20. Morrison BJ, Rubin JE. Carbapenemase Producing Bacteria in the Food Supply Escaping Detection. *PLOS ONE*. 2015;10(5):e0126717. doi:10.1371/journal.pone.0126717

21. Dolejska M, Literak I. Wildlife Is Overlooked in the Epidemiology of Medically Important Antibiotic-Resistant Bacteria. *Antimicrob Agents Chemother*. 2019;63(8):e01167-19. doi:10.1128/AAC.01167-19

22. World Health Organisation. *Global Antimicobial Resistance Surveillance System (GLASS) Report: Early Implememntaion 2017-2018*.; 2018. https://apps.who.int/iris/bitstream/handle/10665/279656/9789241515061-eng.pdf.

Accessed August 1, 2019.

23. Van Boeckel TP, Brower C, Gilbert M, et al. Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci*. 2015;112(18):5649–5654. doi:10.1073/pnas.1503141112

24. Burow E, Simoneit C, Tenhagen BA, Käsbohrer A. Oral antimicrobials increase antimicrobial resistance in porcine *E. coli*- systematic review. *Prev Vet Med*. 2014;113(4):364–375. doi:10.1016/j.prevetmed.2013.12.007

25. Young I, Rajić A, Wilhelm BJ, Waddell L, Parker S, McEwen SA. Comparison of the prevalence of bacterial enteropathogens, potentially zoonotic bacteria and bacterial resistance to antimicrobials in organic and conventional poultry, swine and beef production: A systematic review and meta-analysis. *Epidemiol Infect*. 2009;137(9):1217–1232. doi:10.1017/S0950268809002635

26. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2012)*.; 2013. https://www.danmap.org/downloads/reports.aspx.

27. SWEDRES-SVARM. *Consumption of Antibiotics and Occurrence of Antibiotic Resistance in Sweden (2015)*.; 2016. https://www.sva.se/en/antibiotika/svarm-reports.

28. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2012)*.; 2014. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

29. Krishnasamy V, Otte J, Silbergeld E. Antimicrobial use in Chinese swine and broiler poultry production. *Antimicrob Resist Infect Control*. 2015;4(1):1–9. doi:10.1186/s13756-015-0050-y

30. Collignon P, Powers JH, Chiller TM, Aidara-Kane A, Aarestrup FM. World Health Organization Ranking of Antimicrobials According to Their Importance in Human Medicine: A Critical Step for Developing Risk Management Strategies for the Use of Antimicrobials in Food Production Animals. *Clin Infect Dis*. 2009;49(1):132–141. doi:10.1086/599374

31. Zhao S, Blickenstaff K, Glenn A, et al. β-Lactam resistance in *Salmonella* strains isolated from retail meats in the United States by the national antimicrobial resistance monitoring system between 2002 and 2006. *Appl Environ Microbiol*. 2009;75(24):7624–7630. doi:10.1128/AEM.01158-09

32. Casella T, Correa M, Nogueira L, Saras E, Haenni M, Madec JY. High prevalence of ESBLs in retail chicken meat despite reduced use of antimicrobials in chicken production, France High prevalence of ESBLs in retail chicken meat despite

reduced use of antimicrobials in chicken production. *Int J Food Microbiol*. 2017;257:271–275. doi:10.1016/j.ijfoodmicro.2017.07.005

33. Giske CG, Sundsfjord AS, Kahlmeter G, et al. Redefining extended-spectrum β-lactamases: Balancing science and clinical need. *J Antimicrob Chemother*. 2009;63(1):1–4. doi:10.1093/jac/dkn444

34. Palzkill T. Structural and Mechanistic Basis for Extended-Spectrum Drug-Resistance Mutations in Altering the Specificity of TEM, CTX-M, and KPC β-lactamases. *Front Mol Biosci*. 2018;5:16. doi:10.3389/fmolb.2018.00016

35. Hooper DC, Jacoby GA. Mechanisms of drug resistance: Quinolone resistance. *Ann N Y Acad Sci*. 2015;1354(1):12-31. doi:10.1111/nyas.12830

36. Jacoby GA, Gacharna N, Black TA, Miller GH, Hooper DC. Temporal Appearance of Plasmid-Mediated Quinolone Resistance Genes. *Antimicrob Agents Chemother*. 2009;53(4):1665-1666. doi:10.1128/AAC.01447-08

37. Tadesse DA, Zhao SH, Tong E, et al. Antimicrobial drug resistance in *Escherichia coli* from humans and food animals, United States, 1950-2002. *Emerg Infect Dis*. 2012;18(5):741–749. doi:10.3201/eid1805.111153

38. Marshall BM, Levy SB. Food Animals and Antimicrobials: Impacts on Human Health. *Clin Microbiol Rev*. 2011;24(4):718–733. doi:10.1128/CMR.00002-11

39. World Bank. *World Bank Country and Lending Groups*. https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups. Accessed August 1, 2019.

40. Freeman MF, Tukey JW. Transformations Related to the Angular and the Square Root. *Ann Math Stat*. 1950;21(4):607–611. doi:10.1214/aoms/1177729756

41. EFSA/ECDC. The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2015. *EFSA J*. 2017;15(2):1–212.

42. Viechtbauer W. Conducting Meta-Analyses in R with the metafor Package. *J Stat Softw*. 2010;36(3):1–48. doi:10.1103/PhysRevB.91.121108

43. Berkey CS, Hoaglin DC, Mosteller F, Colditz GA. A random-effects regression model for meta-analysis. *Stat Med*. 1995;14(4):395–411. doi:10.1002/sim.4780140406

44. Pulss S, Semmler T, Prenger-berninghoff E, Bauerfeind R, Ewers C. First report of an *Escherichia coli* strain from swine carrying an OXA-181 carbapenemase and the colistin resistance determinant *mcr-1*. *Int J Antimicrob Agents*. 2017;50(2):232–236. doi:10.1016/j.ijantimicag.2017.03.014

45. Silva KC, Moreno M, Cabrera C, et al. First Characterization of CTX-M-15-Producing Escherichia coli Strains Belonging to Sequence Type (ST) 410, ST224, and ST1284 from Commercial Swine in South America. *Antimicrob Agents Chemother*. 2016;60(4):2505–2508. doi:10.1128/AAC.02788-15

46. Fischer J, Hille K, Ruddat I, Mellmann A, K??ck R, Kreienbrock L. Simultaneous occurrence of MRSA and ESBL-producing Enterobacteriaceae on pig farms and in nasal and stool samples from farmers. *Vet Microbiol*. 2017;200:107–113. doi:10.1016/j.vetmic.2016.05.021

47. García-Cobos S, Köck R, Mellmann A, Frenzel J, Friedrich AW, Rossen JWA. Molecular typing of Enterobacteriaceae from pig holdings in North-Western Germany reveals extended- spectrum and AmpC β-lactamases producing but no carbapenem resistant ones. *PLoS ONE*. 2015;10(7):1–12. doi:10.1371/journal.pone.0134533

48. Changkaew K, Intarapuk A, Utrarachkij F, Nakajima C, Suthienkul O, Suzuki Y. Antimicrobial Resistance, Extended-Spectrum β-Lactamase Productivity, and Class 1 Integrons in *Escherichia coli* from Healthy Swine. *J Food Prot*. 2015;78(8):1442–1450. doi:10.4315/0362-028X.JFP-14-445

49. Han JW, Koh HB, Kim TJ. Molecular Characterization of beta-Lactamase-Producing *Escherichia coli* Collected from 2001 to 2011 from Pigs in Korea. *FOODBORNE Pathog Dis*. 2016;13(2):68–76. doi:10.1089/fpd.2015.2017

50. Hinenoya A, Tran STT, Nguyen NT, et al. Isolation and molecular characterization of extended-spectrum beta -lactamase producing *Escherichia coli* from industrial food animals in Mekong Delta, Vietnam. *Jpn J Vet Res*. 2018;66(1):1–12.

51. Norizuki C, Kawamura K, Wachino JI, et al. Detection of *Escherichia coli* Producing CTX-M-1-Group Extended-Spectrum beta-Lactamases from Pigs in Aichi Prefecture, Japan, between 2015 and 2016. *Jpn J Infect Dis*. 2018;71(1):33–38. doi:10.7883/yoken.JJID.2017.206

52. Nuangmek A, Rojanasthien S, Chotinun S, et al. Antimicrobial Resistance in ESBL-Producing *Escherichia coli* Isolated from Layer and Pig Farms in Thailand. *ACTA Sci Vet*. 2018;46:1538.

53. Delannoy S, le Devendec L, Jouy E, Fach P, Drider D, Kempf I. Characterization of Colistin-Resistant *Escherichia coli* Isolated from Diseased Pigs in France. *Front Microbiol*. 2017;8:2278. doi:10.3389/fmicb.2017.02278

54. Tian GB, Wang HN, Zhang AY, et al. Detection of clinically important β-lactamases in commensal *Escherichia coli* of human and swine origin in western China. *J Med Microbiol*. 2012;61(2):233–238. doi:10.1099/jmm.0.036806-0

55. Roschanski N, Friese A, von Salviati-Claudius C, et al. Prevalence of

carbapenemase producing Enterobacteriaceae isolated from German pig-fattening farms during the years 2011–2013. *Vet Microbiol*. 2017;200(SI):124–129. doi:10.1016/j.vetmic.2015.11.030

56. Tang X, Tan C, Zhang X, et al. Antimicrobial resistances of extraintestinal pathogenic *Escherichia coli* isolates from swine in China. *Microb Pathog*. 2011;50(5):207–212. doi:10.1016/j.micpath.2011.01.004

57. Jakobsen L, Kurbasic A, Skjot-Rasmussen L, et al. *Escherichia coli* isolates from broiler chicken meat, broiler chickens, pork, and pigs share phylogroups and antimicrobial resistance with community-dwelling humans and patients with urinary tract infection. *Foodborne Pathog Dis*. 2010;7(5):537–547. doi:10.1089/fpd.2009.0409

58. Johnson TJ, Shepard SM, Rivet B, Danzeisen JL, Carattoli A. Comparative genomics and phylogeny of the IncI1 plasmids: A common plasmid type among porcine enterotoxigenic *Escherichia coli*. *Plasmid*. 2011;66(3):144–151. doi:10.1016/j.plasmid.2011.07.003

59. Fischer J, Rodriguez I, Schmoger S, et al. *Escherichia coli* producing VIM-1 carbapenemase isolated on a pig farm. *J Antimicrob Chemother*. 2012;67(7):1793–1795. doi:10.1093/jac/dks108

60. Lin D, Xie M, Li R, Chen K, Chan EWC, Chen S. IncFII Conjugative Plasmid-Mediated Transmission of blaNDM-1 Elements among Animal-Borne *Escherichia coli* Strains. *Antimicrob Agents Chemother*. 2017;61(1):e02285–16.

61. Ho P, Wang Y, Liu MC, et al. IncX3 Epidemic Plasmid Carrying blaNDM-5 in *Escherichia coli* from Swine in Multiple Geographic Areas in China. *Antimicrob Agents Chemother*. 2018;62(3):e02295–17.

62. Duggett NA, Sayers E, AbuOun M, et al. Occurrence and characterization of *mcr-1*-harbouring *Escherichia coli* isolated from pigs in Great Britain from 2013 to 2015. *J Antimicrob Chemother*. 2017;72(3):691–695. doi:10.1093/jac/dkw477

63. Alba P, Leekitcharoenphon P, Franco A, et al. Molecular Epidemiology of *mcr*-Encoded Colistin Resistance in Enterobacteriaceae From Food-Producing Animals in Italy Revealed Through the EU Harmonized Antimicrobial Resistance Monitoring. *Front Microbiol*. 2018;9. doi:10.3389/fmicb.2018.01217

64. Xu G, An W, Wang H, Zhang X. Prevalence and characteristics of extended-spectrum β-lactamase genes in *Escherichia coli* isolated from piglets with post-weaning diarrhea in Heilongjiang province, China. *Front Microbiol*. 2015;6. doi:10.3389/fmicb.2015.01103

65. Li R, Xie M, Zhang J, et al. Genetic characterization of *mcr-1*-bearing plasmids to depict molecular mechanisms underlying dissemination of the colistin resistance

determinant. *J Antimicrob Chemother*. 2017;72(2):393–401. doi:10.1093/jac/dkw411

66. Kuo HC, Wei HW, Chang CD, et al. Molecular detection of florfenicol and chloramphenicol resistance among *Escherichia coli* isolates from healthy pigs during 2003 to 2007. *J Food Drug Anal*. 2009;17(3):217–224.

67. Yassin AK, Zhang J, Wang J, et al. Identification and characterization of *mcr* mediated colistin resistance in extraintestinal *Escherichia coli* from poultry and livestock in China. *FEMS Microbiol Lett*. 2018;364(24):fncx242. doi:10.1093/femsle/fnx242

68. Kawanishi M, Abo H, Ozawa M, Uchiyama M. Prevalence of Colistin Resistance Gene *mcr-1* and Absence of *mcr-2* in *Escherichia coli* Isolated from Healthy. *Antimicrob Agents Chemother*. 2017;61(1):e02057–16. doi:10.1128/AAC.02057-16

69. García V, García-meniño I, Mora A, et al. Co-occurrence of *mcr-1, mcr-4* and *mcr-5* genes in multidrug-resistant ST10 Enterotoxigenic and Shiga toxin-producing *Escherichia coli* in Spain (2006-2017). *Int J Antimicrob Agents*. 2018;52:104–108. doi:10.1016/j.ijantimicag.2018.03.022

70. Wang Q, Li Z, Lin J, Wang X, Deng X, Feng Y. Complex dissemination of the diversified *mcr-1* -harbouring plasmids in *Escherichia coli* of different sequence types. *Oncotarget*. 2016;7(50):82112–82122. doi:10.18632/oncotarget.12621

71. Hille K, Roschanski N, Ruddat I, Woydt J, Hartmann M. Investigation of potential risk factors for the occurrence of *Escherichia coli* isolates from German fattening pig farms harbouring the *mcr-1* colistin – resistance gene. *Int J Antimicrob Agents*. 2018;51(2):177–180. doi:10.1016/j.ijantimicag.2017.08.007

72. Li X, Liu B, Dong P, Li F, Yuan L, Hu G. The prevalence of *mcr -1* and resistance characteristics of *Escherichia coli* isolates from diseased and healthy pigs. *Diagn Microbiol Infect Dis*. 2018;91(1):63-65. doi:10.1016/j.diagmicrobio.2017.12.014

73. Xavier BB, Lammens C, Butaye P, Goossens H, Malhotra-Kumar S. Complete sequence of an IncFII plasmid harbouring the colistin resistance gene *mcr-1* isolated from Belgian pig farms. *J Antimicrob Chemother*. 2016;71(8):2342–2344. doi:10.1093/jac/dkw191

74. Kieffer N, Aires-de-Sousa M, Nordmann P, Poirel L. High Rate of *mcr-1*-Producing *Escherichia coli* and *Klebsiella pneumoniae* among Pigs, Portugal. *Emerg Infect Dis*. 2017;23(12):2023–2029. doi:10.3201/eid2312.170883

75. Skočková A, Koláčková I, Kubelová M, Karpíšková R. Shiga toxin-producing *Escherichia coli* (STEC) in the Czech Republic: Characterization of pathogenic strains isolated from pig and cattle carcasses. *J Food Nutr Res*. 2017;56(4):362–

371.

76. Skočková A, Koláčková I, Bogdanovičová K, Karpíšková R. Characteristic and antimicrobial resistance in *Escherichia coli* from retail meats purchased in the Czech Republic. *Food Control*. 2015;47:401–406. doi:10.1016/j.foodcont.2014.07.034

77. Smith MG, Jordan D, Gibson JS, et al. Phenotypic and genotypic profiling of antimicrobial resistance in enteric *Escherichia coli* communities isolated from finisher pigs in Australia. *Aust Vet J*. 2016;94(10):371–376. doi:10.1111/avj.12491

78. Buranasinsup S, Kulpeanprasit S, Kong-ngoen T, et al. Prevalence of the Multi-drug Resistance of Shiga Toxin-producing *Escherichia coli* Isolated from Pigs in Central Thailand. *Chiang Mai J Sci*. 2018;45(1):21–32.

79. Nguyen NT, Nguyen HM, Nguyen CV, et al. Use of colistin and other critical antimicrobials on pig and chicken farms in southern Vietnam and its association with resistance in commensal *Escherichia coli* bacteria. *Appl Environ Microbiol*. 2016;82(13):3727–3735.

80. Zhao J, Chen Z, Chen S, et al. Prevalence and Dissemination of *oqxAB* in *Escherichia coli* Isolates from Animals, Farmworkers, and the Environment. *Antimicrob Agents Chemother*. 2010;54(10):4219-4224. doi:10.1128/AAC.00139-10

81. Chen X, Zhang W, Pan W, et al. Prevalence of *qnr* , *aac(6 ')-Ib-cr* , *qepA* , and *oqxAB* in *Escherichia coli* Isolates from Humans, Animals, and the Environment. *Antimicrob Agents Chemother*. 2012;56(6):3423-3427. doi:10.1128/AAC.06191-11

82. Huang S-Y, Zhu X-Q, Wang Y, et al. Co-carriage of *qnrS1* , *floR* , and *bla* CTX-M-14 on a Multidrug-Resistant Plasmid in *Escherichia coli* Isolated from Pigs. *Foodborne Pathog Dis*. 2012;9(10):896-901. doi:10.1089/fpd.2012.1131

83. Liu BT, Liao X-, Yang SS, et al. Detection of mutations in the *gyrA* and *parC* genes in *Escherichia coli* isolates carrying plasmid-mediated quinolone resistance genes from diseased food-producing animals. *J Med Microbiol*. 2012;61(Pt_11):1591-1599. doi:10.1099/jmm.0.043307-0

84. Yang T, Zeng Z, Rao L, et al. The association between occurrence of plasmid-mediated quinolone resistance and ciprofloxacin resistance in *Escherichia coli* isolates of different origins. *Vet Microbiol*. 2014;170(1-2):89-96. doi:10.1016/j.vetmic.2014.01.019

85. Yeh JC, Lo DY, Chang SK, Chou CC, Kuo HC. Prevalence of plasmid-mediated quinolone resistance in *Escherichia coli* isolated from diseased animals in Taiwan. *J Vet Med Sci*. 2017;79(4):730-735. doi:10.1292/jvms.16-0463

86. Zhang H, Zhao X, Wang X, Chang W. Prevalence and antimicrobial resistance profiles of Escherichia coli isolated from free-range pigs. *J Infect Dev Ctries*. 2017;11(8):652–655. doi:10.3855/jidc.9269

87. Lyu W. Detection of Plasmid-Mediated Quinolone Resistance Genes in Swine Clinical Isolates of *Escherichia coli*. *Pak Vet J*. 2018;38(01):86-90. doi:10.29261/pakvetj/2018.017

88. Hu YS, Shin S, Park YH, et al. Prevalence and Mechanism of Fluoroquinolone Resistance in *Escherichia coli* isolated from Swine Feces in Korea. *J Food Prot*. 2017;80(7):1145–1151. doi:10.4315/0362-028X.JFP-16-502

89. Tamang MD, Nam HM, Chae MH, et al. Prevalence of Plasmid-Mediated Quinolone Resistance Determinants Among *Escherichia coli* Isolated from Food Animals in Korea. *Foodborne Pathog Dis*. 2012;9(12):1057-1063. doi:10.1089/fpd.2012.1225

90. Cavaco LM, Frimodt-Møller N, Hasman H, Guardabassi L, Nielsen L, Aarestrup FM. Prevalence of Quinolone Resistance Mechanisms and Associations to Minimum Inhibitory Concentrations in Quinolone-Resistant *Escherichia coli* Isolated from Humans and Swine in Denmark. *Microb Drug Resist*. 2008;14(2):163-169. doi:10.1089/mdr.2008.0821

91. Zhang WH, Ren SQ, Gu XX, et al. High frequency of virulence genes among *Escherichia coli* with the *bla*$_{\text{CTX-M}}$ genotype from diarrheic piglets in China. *Vet Microbiol*. 2015;180(3-4):260–267. doi:10.1016/j.vetmic.2015.08.017

92. Alonso CA, Zarazaga M, Ben Sallem R, Jouini A, Ben Slama K, Torres C. Antibiotic resistance in *Escherichia coli* in husbandry animals: The African perspective. *Lett Appl Microbiol*. 2017;64(5):318–334. doi:10.1111/lam.12724

93. Roca I, Akova M, Baquero F, et al. The global threat of antimicrobial resistance: Science for intervention. *New Microbes New Infect*. 2015;6:22–29. doi:10.1016/j.nmni.2015.02.007

94. Agerso Y, Aarestrup FM. Voluntary ban on cephalosporin use in Danish pig production has effectively reduced extended-spectrum cephalosporinase-producing *Escherichia coli* in slaughter pigs. *J Antimicrob Chemother*. 2013;68(3):569–572. doi:10.1093/jac/dks427

95. Aarestrup FM. Veterinary drug usage and antimicrobial resistance in bacteria of animal origin. *Basic Clin Pharmacol Toxicol*. 2005;96(4):271–281. doi:10.1111/j.1742-7843.2005.pto960401.x

96. Bush K, Jacoby GA. Updated functional classification of β-lactamases. *Antimicrob Agents Chemother*. 2010;54(3):969–976. doi:10.1128/AAC.01009-09

97. Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi J Biol Sci*. 2015;22(1):90–101. doi:10.1016/j.sjbs.2014.08.002

98. Pires J, Taracila M, Bethel CR, et al. In Vivo Evolution of CMY-2 to CMY-33 β-Lactamase in *Escherichia coli* Sequence Type 131: Characterization of an Acquired Extended- Spectrum AmpC Conferring Resistance to Cefepime. *Antimicrob Agents Chemother*. 2015;59(12):7483–7488. doi:10.1128/AAC.01804-15.Address

99. Mroczkowska JE, Barlow M. Fitness trade-offs in *bla*$_{\text{TEM}}$ evolution. *Antimicrob Agents Chemother*. 2008;52(7):2340–2345. doi:10.1128/AAC.00018-08

100. Liakopoulos A, Mevius D, Ceccarelli D. A review of SHV extended-spectrum β-lactamases: Neglected yet ubiquitous. *Front Microbiol*. 2016;7(SEP). doi:10.3389/fmicb.2016.01374

101. Poirel L, He C, Podglajen I, Sougakoff W, Gutmann L, Nordmann P. Emergence in Klebsiella pneumoniae of a Chromosome-Encoded SHV β-Lactamase That Compromises the Efficacy of Imipenem. *Antimicrob Agents Chemother*. 2003;47(2):755–758. doi:10.1128/AAC.47.2.755

102. Cantón R, González-Alba JM, Galán JC. CTX-M enzymes: Origin and diffusion. *Front Microbiol*. 2012;3(APR). doi:10.3389/fmicb.2012.00110

103. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: Temporal and geographical shifts in genotype. *J Antimicrob Chemother*. 2017;72(8):2145–2155. doi:10.1093/jac/dkx146

104. Dorado-García A, Mevius DJ, Jacobs JJH, et al. Quantitative assessment of antimicrobial resistance in livestock during the course of a nationwide antimicrobial use reduction in the Netherlands. *J Antimicrob Chemother*. 2016;71(12):3607–3619. doi:10.1093/jac/dkw308

105. Muloi D, Ward MJ, Pedersen AB, Fè EM, Woolhouse MEJ, Van Bunnik BAD. Are Food Animals Responsible for Transfer of Antimicrobial-Resistant *Escherichia coli* or Their Resistance Determinants to Human Populations? A Systematic Review. *Foodborne Pathog Dis*. 2018; 15(8): 467-474. doi:10.1089/fpd.2017.2411

106. Woodford N, Wareham DW, Guerra B, Teale C. Carbapenemase-producing enterobacteriaceae and non-enterobacteriaceae from animals and the environment: An emerging public health risk of our own making? *J Antimicrob Chemother*. 2014;69(2):287–291. doi:10.1093/jac/dkt392

107. Nordmann P, Dortet L, Poirel L. Carbapenem resistance in Enterobacteriaceae: Here is the storm! *Trends Mol Med*. 2012;18(5):263–272.

doi:10.1016/j.molmed.2012.03.003

108. Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL. Carbapenemase-producing Enterobacteriaceae in Europe: Assessment by national experts from 38 countries, May 2015. *Eurosurveillance*. 2015;20(45):30062. doi:10.2807/1560-7917.ES.2015.20.45.30062

109. Madec JY, Haenni M, Nordmann P, Poirel L. Extended-spectrum β-lactamase/AmpC- and carbapenemase-producing Enterobacteriaceae in animals: A threat for humans? *Clin Microbiol Infect*. 2017;23(11):826–833. doi:10.1016/j.cmi.2017.01.013

110. Al-Tawfiq JA, Laxminarayan R, Mendelson M. How should we respond to the emergence of plasmid-mediated colistin resistance in humans and animals? *Int J Infect Dis*. 2017;54:77–84. doi:10.1016/j.ijid.2016.11.415

111. Kempf I, Jouy E, Chauvin C. Colistin use and colistin resistance in bacteria from animals. *Int J Antimicrob Agents*. 2016;48(6):598-606.

112. Skov RL, Monnet DL. Plasmid-mediated colistin resistance (*mcr-1* gene): Three months later, the story unfolds. *Eurosurveillance*. 2016;21(9):30155.

113. Rhouma M, Beaudry F, Letellier A. Resistance to colistin: What is the fate for this antibiotic in pig production? *Int J Antimicrob Agents*. 2016;48(2):119–126. doi:10.1016/j.ijantimicag.2016.04.008

114. Ball P. Quinolone generations: Natural history or natural selection? *J Antimicrob Chemother*. 2000;46(suppl_3):17-24. doi:10.1093/oxfordjournals.jac.a020889

115. Trautner BW. Fluoroquinolones for urinary tract infection and within-household spread of resistant Enterobacteriaceae: The smoking gun. *Clin Microbiol Infect*. 2018;24(9):929-930. doi:10.1016/j.cmi.2018.03.038

116. El Garch F, de Jong A, Simjee S, et al. Monitoring of antimicrobial susceptibility of respiratory tract pathogens isolated from diseased cattle and pigs across Europe, 2009–2012: VetPath results. *Vet Microbiol*. 2016;194:11-22. doi:10.1016/j.vetmic.2016.04.009

117. European Medicines Agency, European Surveillance of Veterinary Antimicrobial Consumption. Sales of veterinary antimicrobial agents in 29 European countries in 2014. https://www.ema.europa.eu/en/documents/report/sixth-esvac-report-sales-veterinary-antimicrobial-agents-29-european-countries-2014_en.pdf. Accessed 20 August, 2019.

118. Cheng AC, Turnidge J, Collignon P, Looke D, Barton M, Gottlieb T. Control of Fluoroquinolone Resistance through Successful Regulation, Australia. *Emerg Infect Dis*. 2012;18(9):1453-1460. doi:10.3201/eid1809.111515

119. ECDC/EFSA/EMA. Second joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. EFSA Journal. doi:10.2903/j.efsa.2017.4872

120. Bai L, Lan R, Zhang X, et al. Prevalence of *Salmonella* Isolates from Chicken and Pig Slaughterhouses and Emergence of Ciprofloxacin and Cefotaxime Co-Resistant *S. enterica* Serovar Indiana in Henan, China. Atapattu DN, ed. *PLOS ONE*. 2015;10(12):e0144532. doi:10.1371/journal.pone.0144532

121. Bajaj P, Kanaujia PK, Singh NS, Sharma S, Kumar S, Virdi JS. Quinolone co-resistance in ESBL- or AmpC-producing *Escherichia coli* from an Indian urban aquatic environment and their public health implications. *Environ Sci Pollut Res*. 2016;23(2):1954-1959. doi:10.1007/s11356-015-5609-x

122. Niero G, Bortolaia V, Vanni M, Intorre L, Guardabassi L, Piccirillo A. High diversity of genes and plasmids encoding resistance to third-generation cephalosporins and quinolones in clinical *Escherichia coli* from commercial poultry flocks in Italy. *Vet Microbiol*. 2018;216:93-98. doi:10.1016/j.vetmic.2018.02.012

123. Schar D, Sommanustweechai A, Laxminarayan R, Tangcharoensathien V. Surveillance of antimicrobial consumption in animal production sectors of low- and middle-income countries: Optimizing use and addressing antimicrobial resistance. *PLoS Med*. 2018;15(3):1–9. doi:10.1371/journal.pmed.1002521

124. Goutard FL, Bordier M, Calba C, et al. Antimicrobial policy interventions in food animal production in South East Asia. *Bmj*. 2017;358:j3544. doi:10.1136/bmj.j3544

125. Bessone FA, Bessone G, Marini S, Conde MB, Alustiza FE, Zielinski G. Presence and characterization of *Escherichia coli* virulence genes isolated from diseased pigs in the central region of Argentina. *Vet World*. 2017;10(8):939–945. doi:10.14202/vetworld.2017.939-945

126. Torres Luque A, Gonzalez C, Pasteris SE, Orden JA, De R, Otero MC. Antimicrobial resistant *Escherichia coli* in the reproductive tract microbiota of cows and sows. *Comp Immunol Microbiol Infect Dis*. 2017;55:13–19. doi:10.1016/j.cimid.2017.09.002

127. van Breda LK, Dhungyel OP, Ward MP. Antibiotic resistant *Escherichia coli* in southeastern Australian pig herds and implications for surveillance. *Zoonoses Public Health*. 2018;65(1):E1–E7. doi:10.1111/zph.12402

128. Abraham S, Jordan D, Wong HS, et al. First detection of extended-spectrum cephalosporin- and fluoroquinolone-resistant *Escherichia coli* in Australian food-producing animals. *J Glob Antimicrob Resist*. 2015;3(4):273–277.

doi:10.1016/j.jgar.2015.08.002

129. Stephens CP. Surveillance for antibiotic resistance in veterinary pathogens from the perspective of a regional diagnostic laboratory. *Commun Dis Intell*. 2003;27:S127–131.

130. Smith MG, Jordan D, Chapman TA, et al. Antimicrobial resistance and virulence gene profiles in multi-drug resistant enterotoxigenic *Escherichia coli* isolated from pigs with post-weaning diarrhoea. *Vet Microbiol*. 2010;145(3-4):299–307. doi:10.1016/j.vetmic.2010.04.004

131. Kidsley AK, Abraham S, Bell JM, et al. Antimicrobial Susceptibility of *Escherichia coli* and *Salmonella* spp. Isolates From Healthy Pigs in Australia: Results of a Pilot National Survey. *Front Microbiol*. 2018;9. doi:10.3389/fmicb.2018.01207

132. EFSA/ECDC. The Community Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from animals and food in the European Union in 2008. *EFSA J*. 2010;8(7):1–261.

133. Callens B, Dewulf J, Kronvall G, et al. Antimicrobial resistance surveillance in *Escherichia coli* by using normalized resistance interpretation. *Vet Microbiol*. 2016;197:1–7. doi:10.1016/j.vetmic.2016.10.019

134. Hendriksen RS, Mevius DJ, Schroeter A, et al. Occurrence of antimicrobial resistance among bacterial pathogens and indicator bacteria in pigs in different European countries from year 2002-2004: The ARBAO-II study. *Acta Vet Scand*. 2008;50(1):19.

135. DesRosiers A, Fairbrother JM, Johnson RP, Desautels C, Letellier A, Quessy S. Phenotypic and genotypic characterization of *Escherichia coli* verotoxin-producing isolates from humans and pigs. *J Food Prot*. 2001;64(12):1904–1911.

136. Amezcua R, Friendship RM, Dewey CE, Gyles C, Fairbrother JM. Presentation of postweaning *Escherichia coli* diarrhea in southern Ontario, prevalence of hemolytic *E. coli* serogroups involved, and their antimicrobial resistance patterns. *Can J Vet Res.* 2002;66(2):73–78.

137. Guan S, Xu R, Chen S, Odumeru J, Gyles C. Development of a Procedure for Discriminating among Escherichia coli Isolates from Animal and Human Sources Development of a Procedure for Discriminating among *Escherichia coli* Isolates from Animal and Human Sources. *Appl Environ Microbiol En*. 2002;68(6):2690–2698. doi:10.1128/AEM.68.6.2690-2698.2002.Updated

138. Maynard C, Fairbrother JM, Bekal S, et al. Antimicrobial resistance genes in enterotoxigenic *Escherichia coli* O149:K91 isolates obtained over a 23-year period from pigs. *Antimicrob Agents Chemother*. 2003;47(10):3214–3221.

doi:10.1128/AAC.47.10.3214-3221.2003

139. Ngeleka M, Pritchard J, Appleyard G, Middleton DM, Fairbrother JM. Isolation and Association of *Escherichia coli* AIDA-I/STb, Rather than EAST1 Pathotype, with Diarrhea in Piglets and Antibiotic Sensitivity of Isolates. *J Vet Diagn Invest*. 2003;15(3):242–252. doi:10.1177/104063870301500305

140. Noamani BN, Fairbrother JM, Gyles CL. Virulence genes of O149 enterotoxigenic *Escherichia coli* from outbreaks of postweaning diarrhea in pigs. *Vet Microbiol*. 2003;97(1-2):87–101. doi:10.1016/j.vetmic.2003.08.006

141. Hariharan H, Coles M, Poole D, Page R. Antibiotic resistance among enterotoxigenic *Escherichia coli* from piglets and calves with diarrhea. *Can Vet J*. 2004;45:605–606.

142. Boerlin P, Travis R, Gyles CL, et al. Antimicrobial resistance and virulence genes of *Escherichia coli* isolates from swine in Ontario. *Appl Environ Microbiol*. 2005;v. 71(11):6753–6761–2005 v.71 no.11. doi:10.1128/AEM.71.11.6753-6761.2005

143. Rosengren LB, Waldner CL, Reid-Smith RJ, Checkley SL, McFall ME, Rajić A. Antimicrobial resistance of fecal *Escherichia coli* isolated from grow-finish pigs in 20 herds in Alberta and Saskatchewan. *Can J Vet Res*. 2008;72(2 SPEC. ISS.):160–167.

144. Akwar HT, Poppe C, Wilson J, et al. Prevalence and patterns of antimicrobial resistance of fecal *Escherichia coli* among pigs on 47 farrow-to-finish farms with different in-feed medication policies in Ontario and British Columbia. (Special Issue: Antimicrobial resistance.). *Can J Vet Res Rev Can Rech Veterinaire*. 2008;72(2):195–201.

145. Rosengren LB, Waldner CL, Reid-Smith RJ, Dowling PM, Harding JCS. Associations between feed and water antimicrobial use in farrow-to-finish swine herds and antimicrobial resistance of fecal *Escherichia coli* from grow-finish pigs. *Microb Drug Resist*. 2007;13(4):261–269. doi:10.1089/mdr.2007.781

146. Varga C, Rajic A, McFall ME, et al. Antimicrobial resistance in generic *Escherichia coli* isolated from swine fecal samples in 90 Alberta finishing farms. *Can J Vet Res.* 2008;72(2, SI):175–180.

147. Kozak GK, Boerlin P, Janecko N, Reid-Smith RJ, Jardine C. Antimicrobial resistance in *Escherichia coli* isolates from swine and wild small mammals in the proximity of swine farms and in natural environments in Ontario, Canada. *Appl Environ Microbiol*. 2009;75(3):559–566. doi:10.1128/AEM.01821-08

148. Deckert A, Gow S, Rosengren L, et al. Canadian integrated program for antimicrobial resistance surveillance (CIPARS) farm program: Results from

finisher pig surveillance. *Zoonoses Public Health*. 2010;57(SUPPL. 1):71–84. doi:10.1111/j.1863-2378.2010.01356.x

149. Sheikh AA, Checkley S, Avery B, et al. Antimicrobial Resistance and Resistance Genes in *Escherichia coli* Isolated from Retail Meat Purchased in Alberta, Canada. *Foodborne Pathog Dis*. 2012;9(7):625–631. doi:10.1089/fpd.2011.1078

150. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2002)*.; 2004. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

151. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2003)*.; 2005. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

152. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2004)*.; 2006. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

153. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2005)*.; 2007. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

154. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2006)*.; 2008. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

155. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2007)*.; 2009. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

156. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2008)*.; 2010. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

157. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2009)*.; 2013. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

158. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2010)*.; 2014. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

159. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2013)*.; 2015. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

160. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2014)*.; 2016. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

161. CIPARS. *Canadian Integrated Program for Antimicrobial Resistance Surveillance Annual Report (2015)*.; 2017. https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/cipars-reports.html#ar.

162. Yang H, Chen S, White DG, et al. Characterization of Multiple-Antimicrobial-Resistant *Escherichia coli* Isolates from Diseased Chickens and Swine in China. *J Clin Microbiol*. 2004;42(8):3483-3489. doi:10.1128/JCM.42.8.3483-3489.2004

163. Tian G-B, Wang HN, Zou LK, et al. Detection of CTX-M-15, CTX-M-22, and SHV-2 Extended-Spectrum B-Lactamases (ESBLs) in *Escherichia coli* Fecal-Sample Isolates from Pig Farms in China. *Foodborne Pathog Dis*. 2009;6(3):297–304.

164. Wang XM, Jiang HX, Liao XP, et al. Antimicrobial resistance, virulence genes, and phylogenetic background in *Escherichia coli* isolates from diseased pigs. *FEMS Microbiol Lett*. 2010;306(1):15–21. doi:10.1111/j.1574-6968.2010.01917.x

165. Wang X-M, Liao X-P, Liu S-G, et al. Serotypes, Virulence Genes, and Antimicrobial Susceptibility of *Escherichia coli* Isolates from Pigs. *Foodborne Pathog Dis*. 2011;8(6):687-692. doi:10.1089/fpd.2010.0739

166. Li X-T, Wang B, Li J-L, et al. Prevalence and antimicrobial susceptibility of bacterial pathogens isolates from diseased swine in southwest, China. *Afr J Microbiol Res*. 2012;6(7):1579–1583. doi:10.5897/AJMR11.1471

167. Li P, Wu D, Liu K, et al. Investigation of Antimicrobial Resistance in *Escherichia coli* and *Enterococci* Isolated from Tibetan Pigs. Ibekwe AM, ed. *PLoS ONE*. 2014;9(4):e95623. doi:10.1371/journal.pone.0095623

168. Zhang P, Shen Z, Zhang C, et al. Surveillance of antimicrobial resistance among *Escherichia coli* from chicken and swine, China, 2008-2015. *Vet Microbiol*.

2017;203:49–55. doi:10.1016/j.vetmic.2017.02.008

169. Nedbalcova K, Nechvatalova K, Pokludova L, et al. Resistance to selected beta-lactam antibiotics. *Vet Microbiol*. 2014;171:328–336. doi:10.1016/j.vetmic.2014.02.004

170. Rosager WN, Peter NJ, Lind JSE, Svend H, Matthew D, Steen PK. Comparison of antimicrobial resistance in *E. coli* isolated from rectal and floor samples in pens with diarrhoeic nursery pigs in Denmark. *Prev Vet Med*. 2017;147:42–49. doi:10.1016/j.prevetmed.2017.08.007

171. Hancock V, Nielsen EM, Krag L, Engberg J, Klemm P. Comparative analysis of antibiotic resistance and phylogenetic group patterns in human and porcine urinary tract infectious *Escherichia coli*. *APMIS Acta Pathol Microbiol Immunol Scand*. 2009;117:786–790. doi:10.1111/j.1600-0463.2009.02542.x

172. DANMAP. *Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Food and Humans in Denmark (1996)*.; 1997. https://www.danmap.org/downloads/reports.aspx.

173. DANMAP. *Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Food and Humans in Denmark (1997)*.; 1998. https://www.danmap.org/downloads/reports.aspx.

174. DANMAP. *Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Food and Humans in Denmark (1998)*.; 1999. https://www.danmap.org/downloads/reports.aspx.

175. DANMAP. *Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Food and Humans in Denmark (1999)*.; 2000. https://www.danmap.org/downloads/reports.aspx.

176. DANMAP. *Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Food and Humans in Denmark (2000)*.; 2001. https://www.danmap.org/downloads/reports.aspx.

177. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2001)*.; 2002. https://www.danmap.org/downloads/reports.aspx.

178. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2002)*.; 2003. https://www.danmap.org/downloads/reports.aspx.

179. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2003)*.; 2004. https://www.danmap.org/downloads/reports.aspx.

180. DANMAP. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark (2005). 2006. https://www.danmap.org/downloads/reports.aspx.

181. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2004)*.; 2005. https://www.danmap.org/downloads/reports.aspx.

182. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2006)*.; 2007. https://www.danmap.org/downloads/reports.aspx.

183. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2008)*.; 2009. https://www.danmap.org/downloads/reports.aspx.

184. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2009)*.; 2010. https://www.danmap.org/downloads/reports.aspx.

185. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2010)*.; 2011. https://www.danmap.org/downloads/reports.aspx.

186. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2011)*.; 2012. https://www.danmap.org/downloads/reports.aspx.

187. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2013)*.; 2014. https://www.danmap.org/downloads/reports.aspx.

188. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2014)*.; 2015. https://www.danmap.org/downloads/reports.aspx.

189. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2015)*.; 2016. https://www.danmap.org/downloads/reports.aspx.

190. DANMAP. *Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods and Humans in Denmark (2016)*.; 2017. https://www.danmap.org/downloads/reports.aspx.

191. EFSA/ECDC. Antimicrobial resistance in zoonotic and indcator bacteria from animals and food in the European Union in 2004-2007. *EFSA J*. 2010;223(January):1–320.

192. Wu S, Dalsgaard A, Vieira AR, Emborg HD, Jensen LB. Prevalence of tetracycline resistance and genotypic analysis of populations of *Escherichia coli* from animals, carcasses and cuts processed at a pig slaughterhouse. *Int J Food Microbiol*. 2009;135(3):254–259. doi:10.1016/j.ijfoodmicro.2009.07.027

193. Aasmae B, Kalmus P. Antimicrobial Resistance of Animal Pathogens (2006-2009) in Estonia. *Res Rural Dev*. 2012;1:181–187. doi:ISSN 1691-4031

194. FINRES-VET. *Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents (2002-2003)*.; 2004. https://www.evira.fi/elaimet/elainten-terveys-ja-elaintaudit/laakitseminen/antibioottiresistenssin-seuranta/finres-vet-raporit/.

195. FINRES-VET. *Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents (2004)*.; 2005. https://www.evira.fi/elaimet/elainten-terveys-ja-elaintaudit/laakitseminen/antibioottiresistenssin-seuranta/finres-vet-raporit/.

196. FINRES-VET. *Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents (2005-2006)*.; 2007. https://www.evira.fi/elaimet/elainten-terveys-ja-elaintaudit/laakitseminen/antibioottiresistenssin-seuranta/finres-vet-raporit/.

197. FINRES-VET. *Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents (2007-2009)*.; 2011. https://www.evira.fi/elaimet/elainten-terveys-ja-elaintaudit/laakitseminen/antibioottiresistenssin-seuranta/finres-vet-raporit/.

198. Boireau C, Morignat E, Cazeau G, et al. Antimicrobial resistance trends in *Escherichia coli* isolated from diseased food-producing animals in France: A 14-year period time-series study. *Zoonoses Public Health*. 2018;65(1):E86–E94. doi:10.1111/zph.12412

199. von Müffling T, Smaijlovic M, Nowak B, Sammet K, Bülte M, Klein G. Preliminary study of certain serotypes, genetic and antimicrobial resistance profiles of verotoxigenic *Escherichia coli* (VTEC) isolated in Bosnia and Germany from cattle or pigs and their products. *Int J Food Microbiol*. 2007;117(2):185–191. doi:10.1016/j.ijfoodmicro.2006.08.003

200. Evangelopoulou G, Filioussis G, Kritas S, Kantere M, Burriel AR. Isolation and Antimicrobial Testing of *Aeromonas* spp ., *Citrobacter* spp ., *Cronobacter* spp ., *Enterobacter* spp. *Pol J Microbiol*. 2015;64(2):185–188.

201. Minas A, Petridou E, Bourtzi-Chatzopoulou E, et al. Antibiotic resistance in indicator bacteria isolated from cattle and swine in Greece. *J Anim Vet Adv*. 2007;6(3):317–322.

202. Thorsteinsdottir TR, Haraldsson G, Fridriksdottir V, Kristinsson KG, Gunnarsson E. Prevalence and genetic relatedness of antimicrobial-resistant Escherichia coli isolated from animals, foods and humans in Iceland. *Zoonoses Public Health*. 2010;57(3):189–196. doi:10.1111/j.1863-2378.2009.01256.x

203. Kojima A, Asai T, Ishihara K, et al. National monitoring for antimicrobial resistance among indicator bacteria isolated from food-producing animals in Japan. *J Vet Med Sci*. 2009;71(10):1301–1308. doi:10.1292/jvms.001301

204. Kijima-Tanaka M, Ishihara K, Kojima A, et al. A national surveillance of Shiga toxin-producing *Escherichia coli* in food-producing animals in Japan. *J Vet Med Ser B*. 2005;52(5):230–237. doi:10.1111/j.1439-0450.2005.00852.x

205. Makita K, Goto M, Ozawa M, et al. Multivariable analysis of the association between antimicrobial use and antimicrobial resistance *in Escherichia coli* isolated from apparently healthy pigs in Japan. *Microb Drug Resist*. 2016;22(1):28–39.

206. Kijima-Tanaka M, Ishihara K, Morioka A, et al. A national surveillance of antimicrobial resistance in *Escherichia coli* isolated from food-producing animals in Japan. *J Antimicrob Chemother*. 2003;51(2):447–451. doi:10.1093/jac/dkg014

207. Park HE, Shin MK, Park HT, et al. Virulence factors, antimicrobial resistance patterns, and genetic characteristics of hydrogen sulfide-producing *Escherichia coli* isolated from swine. *Korean J Vet Res*. 2015;55(3):191–197.

208. Kang SG, Lee DY, Shin SJ, Ahn JM, Yoo HS. Changes in patterns of antimicrobial susceptibility and class 1 integron carriage among *Escherichia coli* isolates. *J Vet Sci*. 2005;6(3):201–205. doi:200509201

209. Rayamajhi N, Kang SG, Lee DY, et al. Characterization of TEM-, SHV- and AmpC-type β-lactamases from cephalosporin-resistant Enterobacteriaceae isolated from swine. *Int J Food Microbiol*. 2008;124(2):183–187. doi:10.1016/j.ijfoodmicro.2008.03.009

210. Choi CS, Ham HJ, Kwon D, et al. Antimicrobial susceptibility of pathogenic *Escherichia coli* isolated from pigs in Korea. *J Vet Med Sci*. 2002;64(1):71–73. doi:10.1292/jvms.64.71

211. Ruzauskas M, Klimiene I, Zienius D. Survey of antimicrobial susceptibility among some pathogenic and commensal bacteria isolated from pigs in Lithuania. *Med Weter*. 2006;62(4):397–400.

212. NORMVET. *Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway (2002)*.; 2003.

213. NORMVET. *Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway (2001)*.; 2002.

214. NORMVET. *Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway (2004)*.; 2005.

215. Mazurek J, Bok E, Pusz P, Stosik M, Baldy-Chudzik K. Phenotypic and genotypic characteristics of antibiotic resistance of commensal *Escherichia coli* isolates from healthy pigs. *Bull Vet Inst Pulawy*. 2014;58(2):211–218. doi:10.2478/bvip-2014-0031

216. Herman V, Pascu C, Costinar L, et al. *E. coli* strains' characterization isolated from pig septicemic colibacilosis. *Lucr Stiintifice Med Vet Timiosara*. 2010;43(1):93–96.

217. Surpat AS, Pascu C, Cosinar L, et al. *Escherichia coli* strains characterization isolated from post-weaning diarrhea in pigs. *Bull UASVM Vet Med*. 2011;68(2):286–289.

218. Žutic´ J, Radosavljevic´ V, Radanovic´ O, Mrenoski S, Bacic´ D, Žutic´ M. Antimicrobial resistance observed in *Escherichia coli* isolates from pigs. *Arch Zootech*. 2013;16(2):75–82.

219. Mateu E, Martin M. Antimicrobial resistance in enteric porcine *Escherichia coli* strains in Spain. *Vet Rec*. 2000;146(24):703–706.

220. SVARM. *Swedish Veterinary Antimicrobial Resistance Monitoring (2011)*.; 2012. https://www.sva.se/en/antibiotika/svarm-reports.

221. SWEDRES-SVARM. *Consumption of Antibiotics and Occurrence of Antibiotic Resistance in Sweden (2014)*.; 2015. https://www.sva.se/en/antibiotika/svarm-reports.

222. SWEDRES-SVARM. *Use of Antimicrobials and Occurrence of Antimicrobial Resistance in Sweden (2013)*.; 2014. https://www.sva.se/en/antibiotika/svarm-reports.

223. SWEDRES-SVARM. *Use of Antimicrobials and Occurrence of Antimicrobial Resistance in Sweden (2012)*.; 2013. https://www.sva.se/en/antibiotika/svarm-reports.

224. SVARM. *Swedish Veterinary Antimicrobial Resistance Monitoring (2009)*.; 2010. https://www.sva.se/en/antibiotika/svarm-reports.

225. SVARM. *Swedish Veterinary Antimicrobial Resistance Monitoring (2005)*.; 2006. https://www.sva.se/en/antibiotika/svarm-reports.

226. SVARM. *Swedish Veterinary Antimicrobial Resistance Monitoring (2002)*.; 2003. https://www.sva.se/en/antibiotika/svarm-reports.

227. Kuo HC, Chou CC, Tu C, et al. Characterization of plasmid-mediated quinolone resistance by the *qnrS* gene in *Escherichia coli* isolated from healthy chickens and

pigs. *Vet Med (Praha)*. 2009;54(10):473–482.

228. Kramomtong I, Tripipat T, Ayudhya SNN, Tummaruk P, Leangyodleuchakul S. Extended-Spectrum Beta-lactamase Produced by *Escherichia coli* Strains Isolated from Diarrheal Piglets. In: Kanchanapangka, S and Rungsipipat, A and Ingkaninun, P, ed. *15TH Congress of the Federation of Asian veterinary associations, FAVA-OIE Joint Symposium on Emerging Diseases, Proceedings*; 2008: P233—-P234.

229. Ju W, Shen J, Li Y, et al. Non-O157 Shiga toxin-producing *Escherichia coli* in retail ground beef and pork in the Washington D.C. area. *Food Microbiol*. 2012;32(2):371–377. doi:10.1016/j.fm.2012.07.017

230. Xia X, Meng J, Zhao S, et al. Identification and antimicrobial resistance of extraintestinal pathogenic *Escherichia coli* from retail meats. *J Food Prot*. 2011;74(1):38–44. doi:10.4315/0362-028X.JFP-10-251

231. Malik YS, Chander Y, Olsen K, Goyal SM. Antimicrobial resistance in enteric pathogens isolated from Minnesota pigs from 1995 to 2004. *Can J Vet Res*. 2011;75(2):117–121.

232. Alali WQ, Scott HM, Christian KL, et al. Relationship between level of antibiotic use and resistance among *Escherichia coli* isolates from integrated multi-site cohorts of humans and swine. *Prev Vet Med*. 2009;90(3-4):160–167. doi:10.1016/j.prevetmed.2009.05.018

233. Fratamico PM, Bhagwat AA, Injaian L, Fedorka-Cray PJ. Characterization of Shiga Toxin-Producing *Escherichia coli* Strains Isolated from Swine Feces. *Foodborne Pathog Dis*. 2008;5(6):827–838. doi:10.1089/fpd.2008.0147

234. Bunner CA, Norby B, Bartlett PC, Erskine RJ, Downes FP, Kaneene JB. Prevalence and pattern of antimicrobial susceptibility in *Escherichia coli* isolated from pigs reared under anti microbial-free and conventional production methods. *J Am Vet Med Assoc*. 2007;231(2):275–283. doi:10.2460/javma.231.2.275

235. Scott HM, Campbell L, Harvey R, et al. Patterns of Antimicrobial Resistance Among Commensal. *Foodborne Pathog Dis*. 2005;2(1):24–37.

236. Schroeder CM, White DG, Ge B, et al. Isolation of antimicrobial-resistant *Escherichia coli* from retail meats purchased in Greater Washington, DC, USA. *Int J Food Microbiol*. 2003;85(1-2):197–202.

237. Bischoff KM, White DG, McDermott PF, et al. Characterization of chloramphenicol resistance in beta-hemolytic *Escherichia coli* associated with diarrhea in neonatal swine. *J Clin Microbiol*. 2002;40(2):389–394. doi:10.1128/JCM.40.2.389-394.2002

238. Oanh TKN, Nguyen VK, Do TN, Goddeeris BM, de Greve H. *Escherichia coli*

strains causing edema disease in northern Vietnam share an identical verotoxin 2e. *Trop Anim Health Prod*. 2010;42(8):1797–1804. doi:10.1007/s11250-010-9639-6

239. Do N, Cu H, Nguyen N, et al. Antimicrobial resistance phenotypes of ETEC isolates from piglets with diarrhea in North Vietnam. *Ann N Y Acad Sci*. 2006;1081(1):543–545. doi:10.1196/annals.1373.082

240. Abraham S, Trott DJ, Jordan D, et al. Phylogenetic and molecular insights into the evolution of multidrug-resistant porcine enterotoxigenic *Escherichia coli* in Australia. *Int J Antimicrob Agents*. 2014;44(2):105–111. doi:10.1016/j.ijantimicag.2014.04.011

241. EFSA/ECDC. The European Union Summary Report on antimicrobial resistance in zoonotic Antimicrobial resistance in zoonotic and indicator bacteria from humans , animals and food in the European Union in 2012. *EFSA J*. 2014;12(3):1–336. doi:10.2903/j.efsa.2014.3590

242. EFSA/ECDC. The European Union Summary Report on antimicrobial resistance in Antimicrobial resistance in zoonotic and indicator bacteria from humans , animals and food in the European Union in 2011. *EFSA J*. 2013;11(5):1–359. doi:10.2903/j.efsa.2013.3196.Available

243. EFSA/ECDC. The European Union Summary Report on antimicrobial resistance in Antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in the European Union in 2010. *EFSA J*. 2012;10(3):1–2. doi:10.2903/j.efsa.2012.2598.

244. EFSA/ECDC. The European Union Summary Report on antimicrobial resistance in Antimicrobial resistance in zoonotic and indicator bacteria from humans , animals and food in the European Union in 2009. *EFSA J*. 2011;9(7):1–321. doi:10.2903/j.efsa.2011.2154.

245. Chantziaras I, Dewulf J, Boyen F, Callens B, Butaye P. Antimicrobial resistance prevalence of pathogenic and commensal *Escherichia coli* in food-producing animals in Belgium. *Vlaams Diergeneeskd Tijdschr*. 2014;83(5):225–233.

246. Urumova V. Prevalence of resistance to sulfonamides and streptomycin among commensal porcine *Escherichia coli* isolates. *Rev Med Veterinaire*. 2016;167(1-2):38–44.

247. Urumova V. Investigations on tetracycline resistance in commensal *Escherichia coli* isolates from swine. *Bulg J Vet Med*. 2016;19(3):179–188. doi:10.15547/bjvm.992

248. Urumova V. Investigations on the resistance of porcine commensal *E. coli* isolates to beta lactams. *Acta Vet (Beogr)*. 2015;65(4):538–547. doi:10.1515/acve-2015-0045

249. Urumova V, Lyutskanov M, Petrov V. Investigations on the resistance of commensal swine *Escherichia coli* to some aminoglycosides-aminocyclitols. *Arh Vet Med*. 2015;8(1):13–26.

250. Ström G, Boqvist S, Albihn A, et al. Antimicrobials in small-scale urban pig farming in a lower middle-income country – arbitrary use and high resistance levels. *Antimicrob Resist Infect Control*. 2018;7(1):35.

251. Martín BS, Campos L, Adasne M, Borie C. Evaluation of Antimicrobial Resistance Using Indicator Bacteria Isolated From Pigs and Poultry in Chile. *Intern J Appl Res Vet Med*. 2005;3(2):171–178.

252. Li J, Ma Y, Hu C, et al. Dissemination of Cefotaxime-M-Producing *Escherichia coli* Isolates in Poultry Farms, But Not Swine Farms, in China. *Foodborne Pathog Dis*. 2010;7(11):1387–1392.

253. Zheng H, Zeng Z, Chen S, et al. Prevalence and characterisation of CTX-M β-lactamases amongst *Escherichia coli* isolates from healthy food animals in China. *Int J Antimicrob Agents*. 2012;39(4):305-310. doi:10.1016/j.ijantimicag.2011.12.001

254. Meng Q, Bai X, Zhao A, et al. Characterization of Shiga toxin-producing *Escherichia coli* isolated from healthy pigs in China. *BMC Microbiol*. 2014;14(1):5. doi:10.1186/1471-2180-14-5

255. Rao L, Lv L, Zeng Z, et al. Increasing prevalence of extended-spectrum cephalosporin-resistant *Escherichia coli* in food animals and the diversity of CTX-M genotypes during 2003–2012. *Vet Microbiol*. 2014;172(3-4):534-541. doi:10.1016/j.vetmic.2014.06.013

256. Huang X, Yu L, Chen X, et al. High Prevalence of Colistin Resistance and *mcr-1* Gene in *Escherichia coli* Isolated from Food Animals in China. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.00562

257. Habrun B, Kompes G, Cvetnic Z, Spicic S, Benic M, Mitak M. Antimicrobial sensitivity of *Escherichia coli, Salmonella spp., Pasteurella multocida, Streptococcus suis* and *Actinobacillus pleuropneumoniae* isolated from diagnostic samples from large pig breeding farms in Croatian. *Vet Arh*. 2010;80(5):571–583.

258. Habrun B, Stojanovic D, Kompes G, Benic M. Antimicrobial susceptibility of enterotoxigenic strains of *Escherichia coli* isolated from weaned pigs in Croatia. *Acta Vet-Beogr*. 2011;61(5-6):585–590. doi:10.2298/AVB1106585H

259. de la Fé Rodríguez PY, Kiiru JN, Maroto Martin LO, et al. Characterization and clonal grouping of pathogenic *Escherichia coli* isolated from intestinal contents of diarrheic piglets in Villa Clara province, Cuba, according to their antibiotic resistance and ERIC-PCR profiles. *Vet Microbiol*. 2012;154(3-4):425–428.

doi:10.1016/j.vetmic.2011.08.006

260. de Jong A, Thomas V, Simjee S, et al. Pan-European monitoring of susceptibility to human-use antimicrobial agents in enteric bacteria isolated from healthy food-producing animals. *J Antimicrob Chemother*. 2012;67(3):638–651. doi:10.1093/jac/dkr539

261. de Jong A, Bywater R, Butty P, et al. A pan-European survey of antimicrobial susceptibility towards human-use antimicrobial drugs among zoonotic and commensal enteric bacteria isolated from healthy food-producing animals. *J Antimicrob Chemother*. 2009;63(4):733–744. doi:10.1093/jac/dkp012

262. Bywater R, Deluyker H, Deroover E, et al. A European survey of antimicrobial susceptibility among zoonotic and commensal bacteria isolated from food-producing animals. *J Antimicrob Chemother*. 2004;54(4):744–754. doi:10.1093/jac/dkh422

263. Osterberg J, Wingstrand A, Jensen AN, et al. Antibiotic resistance in *Escherichia coli* from pigs in organic and conventional farming in four European countries. *PloS One*. 2016;11(6):e0157049. doi:10.1371/journal.pone.0157049

264. Aasmae B, Kalmus P, Kalmus K, Hakkinen L. Monitoring of antimicrobial resistance of animal pathogens in Estonia. In: *Animal Hygiene and Sustainable Livestock Production. Proceedings of the XVth International Congress of the International Society for Animal Hygiene*. ; 2011:1431–1433.

265. FINRES-VET. *Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents (2013-2015)*.; 2017. https://www.evira.fi/elaimet/elainten-terveys-ja-elaintaudit/laakitseminen/antibioottiresistenssin-seuranta/finres-vet-raporit/.

266. FINRES-VET. *Finnish Veterinary Antimicrobial Resistance Monitoring and Consumption of Antimicrobial Agents (2010-2012)*.; 2015. https://www.evira.fi/elaimet/elainten-terveys-ja-elaintaudit/laakitseminen/antibioottiresistenssin-seuranta/finres-vet-raporit/.

267. Paivarinta M, Pohjola L, Fredriksson-Ahomaa M, Heikinheimo A. Low occurrence of extended-spectrum beta -lactamase-producing *Escherichia coli* in Finnish food-producing animals. *Zoonoses Public Health*. 2016;63(8):624–631.

268. Hering J, Hille K, Fromke C, et al. Prevalence and potential risk factors for the occurrence of cefotaxime resistant *Escherichia coli* in German fattening pig farms-A cross-sectional study. *Prev Vet Med*. 2014;116(1-2):129–137.

269. Kaesbohrer A, Schroeter A, Tenhagen BA, Alt K, Guerra B, Appel B. Emerging antimicrobial resistance in commensal *Escherichia coli* with public health relevance. *Zoonoses Public Health*. 2012;59(SUPPL.2):158–165.

doi:10.1111/j.1863-2378.2011.01451.x

270. Amadi VA, Matthew-Belmar V, Tiwari K, Brathwaite E, Sharma R, Hariharan H. Antimicrobial susceptibility profiles of *Escherichia coli* recovered from feces of young healthy domestic pigs in Grenada, West Indies. *Br Microbiol Res J*. 2015;5(3):300–306.

271. Begum S, Hazarika GC, Rajkhowa S. Prevalence and Antimicrobial Susceptibility Pattern of Shiga Toxin Producing *Escherichia coli* (STEC) from Pigs and Cattle. *Int J Vet Sci*. 2015;4(4):221–223.

272. Dutta PR, Phukan A, Hazarika RA, Rajkhowa S. Isolation, identification, serotyping and antibiogram of *Escherichia coli* from piglets with different health status. *Indian Vet J*. 2016;93(1):38–40.

273. Dubal Z, Rahman H, Murugkar H, et al. Characterization of *Escherichia coli* Isolates from piglet diarrhoea. *Indian J Anim Sci*. 2005;75(12):1388–1389. doi:10.1089/mdr.2011.0032

274. Mandakini R, Dutta TK, Chingtham S, et al. ESBL-producing Shiga-toxigenic *E. coli* (STEC) associated with piglet diarrhoea in India. *Trop Anim Health Prod*. 2015;47(2):377–381.

275. Lalzampuia H, Dutta TK, Warjri I, Chandra R. PCR-Based Detection of Extended-Spectrum β-Lactamases (bla CTX-M-1 and bla TEM) in *Escherichia coli, Salmonella* spp. And *Klebsiella pneumoniae* Isolated from Pigs in North Eastern India (Mizoram). *Indian J Microbiol*. 2013;53(3):291–296. doi:10.1007/s12088-013-0378-z

276. Dutta TK, Roychoudhury P, Bandyopadhyay S, Chandra R. Detection and characterization of shiga toxigenic *Escherichia coli* from piglets with or without diarrhoea in Mizoram. *Indian J Anim Sci*. 2011;81(9):899–903.

277. Kylla H, Passah P. Serogroups and antibiogram profile of *Escherichia coli* isolated from diarrhoeic piglets of Meghalaya. *North-East Vet*. 2015;15(3):38–40.

278. Begum J, Dutta TK, Choudhary PR, Chandra R, Varte Z. Antimicrobial assay of shigatoxigenic E. coli (STEC) and enteropathogenic E. coli (EPEC) isolated from diarrhoeic faecal samples of piglets and infants in Mizoram. *Indian J Anim Sci*. 2015;85(10):1067–1072.

279. Hiroi M, Harada T, Kawamori F, et al. A survey of beta -lactamase-producing *Escherichia coli* in farm animals and raw retail meat in Shizuoka Prefecture, Japan. *Jpn J Infect Dis*. 2011;64:153–155.

280. Lee M, Shin E, Lee Y. Antimicrobial Resistance and Integron Profiles in Multidrug-Resistant *Escherichia coli* Isolates from Pigs. *Foodborne Pathog Dis*.

2014;11(12):988–997. doi:10.1089/fpd.2014.1795

281. Yoo SJ, SunYoung S, SangWon S, Lyoo YS. Comparison of antibiotic resistance profiles for *Escherichia coli* isolated from wild boar and domestic pig fecal samples. *Korean J Vet Res*. 2015;55(1):41–46. doi:10.14405/kjvr.2015.55.1.41

282. Lim SK, Lee HS, Nam HM, et al. Antimicrobial resistance observed in *Escherichia coli* strains isolated from fecal samples of cattle and pigs in Korea during 2003-2004. *Int J Food Microbiol*. 2007;116(2):283–286. doi:10.1016/j.ijfoodmicro.2006.12.014

283. Ho WS, Tan LK, Ooi PT, Yeo CC, Thong KL. Prevalence and characterization of verotoxigenic-*Escherichia coli* isolates from pigs in Malaysia. *BMC Vet Res*. 2013;9(1):678. doi:10.1186/1746-6148-9-109

284. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2015*.; 2016. https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

285. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2016*.; 2017. https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

286. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2014*.; 2015. https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

287. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2009*.; 2009. https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

288. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2006/2007*.; 2007. https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

289. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2013*.; 2014. https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

290. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands (2012)*.; 2012. https://www.wur.nl/nl/Onderzoek-

Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

291. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2005*.; 2005. https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

292. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2004*.; 2004. https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

293. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2003*.; 2003. https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

294. MARAN. *Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2002*.; 2002. https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Publicaties/MARAN-rapporten-antibioticaresistentie.htm.

295. Chikwendu CI, Nwabueze RN, Anyanwu BN. Antibiotic resistance profile of *Escherichia coli* from clinically healthy pigs and their commercial farm environments. *Afr J Microbiol Res*. 2008;2(2):12–17.

296. NORMVET. *Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Animals, Feed and Food in Norway (1999)*.; 1999.

297. Wasyl D, Hoszowski A, Zajac M, Szulowski K. Antimicrobial resistance in commensal *Escherichia coli* isolated from animals at slaughter. *Front Microbiol*. 2013;4:221.

298. Ramos S, Silva N, Caniça M, et al. High prevalence of antimicrobial-resistant *Escherichia coli* from animals at slaughter: A food safety risk. *J Sci Food Agric*. 2013;93(3):517–526. doi:10.1002/jsfa.5814

299. Iwu CJ, Jaja IF, Iweriebor BC, Obi LC, Okoh AI. Antibiotic resistance profiles of *Escherichia coli* O26, O145, and O157:H7 isolated from swine in the Eastern Cape Province, South Africa. *Asian Pac J Trop Dis*. 2017;7(9):553–559.

300. Cabal A, Gomez-Barrero S, Porrero C, et al. Assessment of virulence factors characteristic of human *Escherichia coli* pathotypes and antimicrobial resistance in O157:H7 and non-O157:H7 isolates from livestock in Spain. *Appl Environ Microbiol*. 2013;79(13):4170–4172. doi:10.1128/AEM.00537-13

301. Teshager T, Herrero IA, Porrero MC, Garde J, Moreno MA, Dominguez L. Surveillance of antimicrobial resistance in *Escherichia coli* strains isolated from pigs at Spanish slaughterhouses. *Int J Antimicrob Agents*. 2000;15(2):137–142. doi:10.1016/S0924-8579(00)00153-9

302. Sáenz Y, Zarazaga M, Briñas L, Lantero M, Ruiz-Larrea F, Torres C. Antibiotic resistance in *Escherichia coli* isolates obtained from animals, foods and humans in Spain. *Int J Antimicrob Agents*. 2001;18(4):353–358. doi:10.1016/S0924-8579(01)00422-8

303. SWEDRES-SVARM. *Consumption of Antibiotics and Occurrence of Antibiotic Resistance in Sweden (2016)*.; 2017. https://www.sva.se/en/antibiotika/svarm-reports.

304. Brand P, Gobeli S, Perreten V. Pathotyping and antibiotic resistance of porcine enterovirulent *Escherichia coli* strains from Switzerland (2014-2015). *Schweiz Arch Tierheilkd*. 2017;159(7):373–380. doi:10.17236/sat00120

305. Ström G, Halje M, Karlsson D, et al. Antimicrobial use and antimicrobial susceptibility in *Escherichia coli* on small- and medium-scale pig farms in north-eastern Thailand. *Antimicrob Resist Infect Control*. 2017;6(1):75. doi:10.1186/s13756-017-0233-9

306. Prapasawat W, Intarapuk A, Chompook P, Nakajima C, Suzuki Y, Suthienkul O. Antimicrobial resistance, integron, virulence gene, and multilocus sequencing typing of *Escherichia coli* isolates from postweaning piglets with and without diarrhea. *Southeast Asian J Trop Med Public Health*. 2017;48(5):1042–1055.

307. Randall LP, Lodge MP, Elviss NC, et al. Evaluation of meat, fruit and vegetables from retail stores in five United Kingdom regions as sources of extended-spectrum beta-lactamase (ESBL)-producing and carbapenem-resistant *Escherichia coli*. *Int J Food Microbiol*. 2017;241:283–290. doi:10.1016/j.ijfoodmicro.2016.10.036

308. Cheney TEA, Smith RP, Hutchinson JP, Brunton LA, Pritchard G, Teale CJ. Cross-sectional survey of antibiotic resistance in *Escherichia coli* isolated from diseased farm livestock in England and Wales. *Epidemiol Infect*. 2015;143(12):2653–2659. doi:10.1017/S0950268814003963

309. Zhou H-C, Liu L, Tian Z-F, et al. Prevalence and patterns of antimicrobial resistant of fecal *Escherichia coli* isolates among pigs on eight farrow-to-finish farms and their production environments in Shandong Province, China. *Turk J Vet Anim Sci*. 2012;36(6):718–722. doi:10.3906/vet-1008-446

310. Hakim AS, Abuelnaga ASM, Ezz-Eldeen AM, Bakry MA, Ismail SA. Prevalence of some food poisoning bacteria in local and imported retail pork by-products in Egyptian markets. *Afr J Microbiol Res*. 2015;9(22):1492–1498.

311. Box ATA, Mevius DJ, Schellen P, Verhoef J, Fluit AC. Integrons in *Escherichia coli* from food-producing animals in the Netherlands. *Microb Drug Resist*. 2005;11(1):53–57. doi:10.1089/mdr.2005.11.53

312. Lee SI, Rayamahji N, Lee WJ, et al. Genotypes, antibiogram, and pulsed-field gel electrophoresis profiles of *Escherichia coli* strains from piglets in Korea. *J Vet Diagn Invest*. 2009;21(4):510–516.

313. Unno T, Han D, Jang J, et al. Genotypic and phenotypic trends in antibiotic resistant pathogenic *Escherichia coli* isolated from humans and farm animals in South Korea. *Microbes Environ*. 2011;26(3):198–204.

314. Kang HY, Jeong YS, Oh JY, et al. Characterization of antimicrobial resistance and class 1 integrons found in *Escherichia coli* isolates from humans and animals in Korea. *J Antimicrob Chemother*. 2005;55(5):639–644. doi:10.1093/jac/dki076

315. Omotosho OO, Olaogun SC, Odukaye AO. Occurrence of skin abscess and sensitivity pattern of associated bacterial organisms in pigs on Ifelodun farm settlement, Ogun State, Nigeria. *Alex J Vet Sci*. 2016;51(1):10–16.

316. Hsu S-C, Chiu T-H, Pang J-C, Hsuan-Yuan C-H, Chang G-N, Tsen H-Y. Characterisation of antimicrobial resistance patterns and class I integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. *Int J Antimicrob Agents*. 2006;27(5):383–391. doi:10.1016/j.ijantimicag.2005.11.020

317. Enne VI, Cassar C, Sprigings K, Woodward MJ, Bennett PM. A high prevalence of antimicrobial resistant *Escherichia coli* isolated from pigs and a low prevalence of antimicrobial resistant *E. coli* from cattle and sheep in Great Britain at slaughter. *FEMS Microbiol Lett*. 2008;278(2):193–199. doi:10.1111/j.1574-6968.2007.00991.x

318. Nhung NT, Cuong NV, Campbell J, et al. High Levels of Antimicrobial Resistance among *Escherichia coli* Isolates from Livestock Farms and Synanthropic Rats and Shrews in the Mekong Delta of Vietnam. *Appl Environ Microbiol*. 2015;81(3):812–820. doi:10.1128/AEM.03366-14

319. Trongjit S, Angkittitrakul S, Chuanchuen R. Occurrence and molecular characteristics of antimicrobial resistance of *Escherichia coli* from broilers, pigs and meat products in Thailand and Cambodia provinces. *Microbiol Immunol*. 2016;60(9):575–585. doi:10.1111/1348-0421.12407

320. Chen L, Chen ZL, Liu JH, Zeng ZL, Ma JY, Jiang HX. Emergence of RmtB methylase-producing *Escherichia coli* and *Enterobacter cloacae* isolates from pigs in China. *J Antimicrob Chemother*. 2007;59(5):880-885. doi:10.1093/jac/dkm065

321. Xue Y, Chen J, Hua Y. Resistance of Strains Producing Extended-Spectrum beta-

lactamases and Genotype Distribution Among *Escherichia coli* in China. *Pak J Zool*. 2012;44(2):457–461.

322. Hu YY, Cai JC, Zhou HW, et al. Molecular Typing of CTX-M-Producing *Escherichia coli* Isolates from Environmental Water, Swine Feces, Specimens from Healthy Humans, and Human Patients. *Appl Environ Microbiol*. 2013;79(19):5988-5996. doi:10.1128/AEM.01740-13

323. Zhang H, Zhai Z, Li Q, et al. Characterization of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* Isolates from Pigs and Farm Workers. *J Food Prot*. 2016;79(9):1630-1634. doi:10.4315/0362-028X.JFP-16-093

324. Bardon J, Husickova V, Chroma M, Kolar M. Prevalence and Characteristics of *Escherichia coli* Strains Producing Extended-Spectrum β-Lactamases in Slaughtered Animals in the Czech Republic. *J Food Prot*. 2013;76(10):1773–1777. doi:10.4315/0362-028X.JFP-13-114

325. Wu S, Chouliara E, Hasman H, Dalsgaard A, Vieira A, Jensen LB. Detection of a single isolate of CTX-M-1-producing *Escherichia coli* from healthy pigs in Denmark. *J Antimicrob Chemother*. 2008;61(3):747–749. doi:10.1093/jac/dkm502

326. Michael GB, Kaspar H, Siqueira AK, et al. Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates collected from diseased food-producing animals in the GERM-Vet monitoring program 2008–2014. *Vet Microbiol*. 2017;200:142–150. doi:10.1016/j.vetmic.2016.08.023

327. Schink AK, Kadlec K, Schwarz S. Analysis of blaCTX-M-carrying plasmids from *Escherichia coli* isolates collected in the BfT-GermVet study. *Appl Environ Microbiol*. 2011;77(20):7142–7146. doi:10.1128/AEM.00559-11

328. Schink AK, Kadlec K, Kaspar H, Mankertz J, Schwarz S. Analysis of extended-spectrum- beta -lactamase-producing *Escherichia coli* isolates collected in the GERM-Vet monitoring programme. *J Antimicrob Chemother*. 2013;68(8):1741–1749. doi:10.1093/jac/dkt123

329. Ho PL, Chow KH, Lai EL, et al. Extensive dissemination of CTX-M-producing *Escherichia coli* with multidrug resistance to “critically important” antibiotics among food animals in Hong Kong, 2008-10. *J Antimicrob Chemother*. 2011;66(4):765–768. doi:10.1093/jac/dkq539

330. Samanta I, Joardar SN, Mahanti A, Bandyopadhyay S, Sar TK, Dutta TK. Approaches to characterize extended spectrum beta-lactamase/beta-lactamase producing *Escherichia coli* in healthy organized vis-a-vis backyard farmed pigs in India. *Infect Genet Evol*. 2015;36(October):224–230. doi:10.1016/j.meegid.2015.09.021

331. Lalruatdiki A, Dutta TK, Roychoudhury P, Subudhi PK. Extended-spectrum beta -

lactamases producing multidrug resistance *Escherichia coli, Salmonella* and *Klebsiella pneumoniae* in pig population of Assam and Meghalaya, India. *Vet World*. 2018;11(6):868–873. doi:10.14202/vetworld.2018.868-873

332. Stefani S, Giovanelli I, Anacarso I, et al. Prevalence and characterization of extended-spectrum beta -lactamase-producing Enterobacteriaceae in food-producing animals in Northern Italy. *New Microbiol*. 2014;37(4):551–555.

333. Hiroi M, Yamazaki F, Harada T, et al. Prevalence of extended-spectrum beta -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in food-producing animals. *J Vet Med Sci*. 2012;74(2):189–195. doi:10.1292/jvms.11-0372

334. Hiki M, Usui M, Kojima A, Ozawa M, Ishii Y, Asai T. Diversity of Plasmid Replicons Encoding the blaCMY-2 Gene in Broad-Spectrum Cephalosporin-Resistant *Escherichia coli* from Livestock Animals in Japan. *Foodborne Pathog Dis*. 2013;10(3):243–249. doi:10.1089/fpd.2012.1306

335. Lim S-K, Lee H-S, Nam H-M, Jung S-C, Bae Y-C. CTX-M-Type beta-Lactamase in *Escherichia coli* isolated from sick animals in Korea. *Microb Drug Resist*. 2009;15(2):139–142.

336. Shin SW, Jung M, Won HG, Belaynehe, Kuastros MekonnenYoo HS. Characteristics of Transmissible CTX-M- and CMY-Type β -Lactamase- Producing *Escherichia coli* Isolates Collected from Pig and Chicken Farms in South Korea. *J Microbiol Biotechnol*. 2017;27(9):1716–1723.

337. Dohmen W, Dorado-Garcia A, Bonten MJM, Wagenaar JA, Mevius D, Heederik DJJ. Risk factors for ESBL-producing *Escherichia coli* on pig farms: A longitudinal study in the context of reduced use of antimicrobials. *PLOS ONE*. 2017;12(3):e0174094. doi:10.1371/journal.pone.0174094

338. Akujobi CO, Ogbulie JN, Alisi CS. Occurrence of extended-spectrum beta -lactamases in *Escherichia coli* isolated from piggery farms in Imo State, Nigeria. *World J Microbiol Biotechnol*. 2008;24(10):2167–2170. doi:10.1007/s11274-008-9726-2

339. NORMVET. *Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway (2015)*.; 2016.

340. NORMVET. *Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway (2011)*.; 2012.

341. NORMVET. *Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway (2009)*.; 2010.

342. Wasyl D, Hasman H, Cavaco LM, Aarestrup FM. Prevalence and characterization of cephalosporin resistance in nonpathogenic *Escherichia coli* from food-

producing animals slaughtered in Poland. *Microb Drug Resist*. 2012;18(1):79–82. doi:10.1089/mdr.2011.0033

343. Lalak A, Wasyl D, Zajc M, et al. Mechanisms of cephalosporin resistance in indicator *Escherichia coli* isolated from food animals. *Vet Microbiol*. 2016;194:69–73. doi:10.1016/j.vetmic.2016.01.023

344. Jones-Dias D, Manageiro V, Martins AP, Ferreira E, Canica M. New Class 2 Integron In2-4 Among IncI1-Positive *Escherichia coli* Isolates Carrying ESBL and PMAbeta Genes from Food Animals in Portugal. *Foodborne Pathog Dis*. 2016;13(1):36–39. doi:10.1089/fpd.2015.1972

345. Machado E, Coque TM, Canton R, Sousa JC, Peixe L. Antibiotic resistance integrons and extended-spectrum beta -lactamases among Enterobacteriaceae isolates recovered from chickens and swine in Portugal. *J Antimicrob Chemother*. 2008;62(2):296–302. doi:10.1093/jac/dkn179

346. Ramos S, Silva N, Dias D, et al. Clonal diversity of ESBL-producing *Escherichia coli* in pigs at slaughter level in Portugal. *Foodborne Pathog Dis*. 2013;10(1):74–79. doi:10.1089/fpd.2012.1173

347. Vogt D, Overesch G, Endimiani A, Collaud A, Thomann A, Perreten V. Occurrence and genetic characteristics of third-generation cephalosporin-resistant *Escherichia coli* in Swiss retail meat. *Microb Drug Resist*. 2014;20(5):485–494.

348. Endimiani A, Rossano A, Kunz D, Overesch G, Perreten V. First countrywide survey of third-generation cephalosporin-resistant *Escherichia coli* from broilers, swine, and cattle in Switzerland. *Diagn Microbiol Infect Dis*. 2012;73(1):31–38. doi:10.1016/j.diagmicrobio.2012.01.004

349. Endimiani A, Hilty M, Perreten V. CMY-2-producing *Escherichia coli* in the nose of pigs. *Antimicrob Agents Chemother*. 2012;56(8):4556–4557. doi:10.1128/AAC.00389-12

350. Geser N, Stephan R, Haechler H. Occurrence and characteristics of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. *BMC Vet Res*. 2012;8(1):21. doi:10.1186/1746-6148-8-21

351. Kraemer JG, Pires J, Kueffer M, et al. Prevalence of extended-spectrum β -lactamase-producing Enterobacteriaceae and Methicillin-Resistant *Staphylococcus aureus* in pig farms in Switzerland. *Sci Total Environ*. 2017;603-604:401–405. doi:10.1016/j.scitotenv.2017.06.110

352. Lee WC, Yeh KS. Characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* isolated from fecal samples of piglets with diarrhea in central and southern Taiwan in 2015. *BMC Vet Res*. 2017;13(1):66. doi:10.1186/s12917-017-

0986-7

353. Boonyasiri A, Tangkoskul T, Seenama C, Saiyarin J, Tiengrim S, Thamlikitkul V. Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand. *Pathog Glob Health*. 2014;108(5):235–245. doi:10.1179/2047773214Y.0000000148

354. Randall LP, Lemma F, Rogers JP, Cheney TEA, Powell LF, Teale CJ. Prevalence of extended-spectrum-beta-lactamase-producing *Escherichia coli* from pigs at slaughter in the UK in 2013. *J Antimicrob Chemother*. 2014;69(11):2947–2950. doi:10.1093/jac/dku258

355. Le QP, Ueda S, Nguyen TNH, et al. Characteristics of Extended-Spectrum beta-Lactamase-Producing *Escherichia coli* in Retail Meats and Shrimp at a Local Market in Vietnam. *Foodborne Pathog Dis*. 2015;12(8):719–725. doi:10.1089/fpd.2015.1954

356. El Garch F, de Jong A, Bertrand X, Hocquet D, Sauget M. *mcr-1*- like detection in commensal *Escherichia coli* and *Salmonella* spp. From food-producing animals at slaughter in Europe. *Vet Microbiol*. 2018;213:42–46. doi:10.1016/j.vetmic.2017.11.014

357. Zhang H, Rehman MU, Li K, et al. Antimicrobial Resistance of *Escherichia coli* Isolated from Tibetan Piglets Suffering from White Score Diarrhea. *Pak Vet J*. 2017;37(1):43–46. doi:10.1097/QCO.0b013e3283638104

358. Bai L, Hurley D, Li J, et al. Characterisation of multidrug-resistant Shiga toxin-producing *Escherichia coli* cultured from pigs in China: Co-occurrence of extended-spectrum beta-lactamase- and *mcr-1*-encoding genes on plasmids. *Int J Antimicrob Agents*. 2016;48(4):445–448. doi:10.1016/j.ijantimicag.2016.06.021

359. Jakobsen L, Garneau P, Kurbasic A, et al. Microarray-based detection of extended virulence and antimicrobial resistance gene profiles in phylogroup B2 *Escherichia coli* of human, meat and animal origin. *J Med Microbiol*. 2011;60(10):1502–1511. doi:10.1099/jmm.0.033993-0

360. Perrin-Guyomard A, Bruneau M, Houee P, et al. Prevalence of *mcr-1* in commensal *Escherichia coli* from French livestock, 2007 to 2014. *Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull*. 2016;21(6):1–3.

361. Irrgang A, Roschanski N, Tenhagen BA, et al. Prevalence of *mcr-1* in *E. coli* from livestock and food in Germany, 2010-2015. *PloS One*. 2016;11(7):e0159863. doi:10.1371/journal.pone.0159863

362. Szmolka A, Anjum MF, La Ragione RM, Kaszanyitzky ÉJ, Nagy B. Microarray based comparative genotyping of gentamicin resistant *Escherichia coli* strains from food animals and humans. *Vet Microbiol*. 2012;156(1-2):110-118.

doi:10.1016/j.vetmic.2011.09.030

363. Kusumoto M, Ogura Y, Gotoh Y, Iwata T, Hayashi T, Akiba M. Colistin-Resistant *mcr-1*-Positive Pathogenic Escherichia Coli in Swine, Japan, 2007-2014. Vol 22. Bacterial and Parasitic Disease Research Division, National Institute of Animal Health, National Agriculture and Food Research Organization, 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan.; 2016. doi:10.3201/eid2207.160234

364. Lim SK, Kang HY, Lee K, Moon DC, Lee HS, Jung SC. First detection of the *mcr-1* gene in *Escherichia coli* isolated from livestock between 2013 and 2015 in South Korea. *Antimicrob Agents Chemother*. 2016;60:AAC.01472–16. doi:10.1128/AAC.01472-16

365. Nishino Y, Shimojima Y, Suzuki Y, et al. Detection of the *mcr-1* gene in colistin-resistant *Escherichia coli* from retail meat in Japan. *Microbiol Immunol*. 2017;61(12):554–557. doi:10.1111/1348-0421.12549

366. Lay KK, Koowattananukul C, Chansong N, Chuanchuen R. Antimicrobial resistance, virulence, and phylogenetic characteristics of *Escherichia coli* isolates from clinically healthy swine. *Foodborne Pathog Dis*. 2012;9(11):992–1001. doi:10.1089/fpd.2012.1175

367. Mollenkopf DF, Mirecki JM, Daniels JB, et al. *Escherichia coli* and *Klebsiella pneumoniae* producing CTX-M cephalosporinase from swine finishing barns and their association with antimicrobial use. *Appl Environ Microbiol*. 2013;79(3):1052–1054. doi:10.1128/AEM.03169-12

368. Meinersmann RJ, Ladely SR, Plumblee JR, Cook KL, Thacker E. Prevalence of *mcr-1* in the cecal contents of food animals in the United States. *Antimicrob Agents Chemother*. 2017;61(2):e02244–16. doi:10.1128/AAC.02244-16

369. Delgado-Blas JF, Ovejero CM, Abadia-Patino L, Gonzalez-Zorn B. Coexistence of *mcr-1* and blaNDM-1 in *Escherichia coli* from Venezuela. *Antimicrob Agents Chemother*. 2016;60(10):6356–6358.

370. Van Damme I, Garcia-Graells C, Biasino W, Gowda T, Botteldoorn N, de Zutter L. High abundance and diversity of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in faeces and tonsils of pigs at slaughter. *Vet Microbiol*. 2017;208:190–194. doi:10.1016/j.vetmic.2017.08.009

371. Jahanbakhsh S, Smith MG, Kohan-Ghadr HR, et al. Dynamics of extended-spectrum cephalosporin resistance in pathogenic *Escherichia coli* isolated from diseased pigs in Quebec, Canada. *Int J Antimicrob Agents*. 2016;48(2):194–202. doi:10.1016/j.ijantimicag.2016.05.001

372. Gao L, Tan Y, Zhang X, et al. Emissions of *Escherichia coli* Carrying Extended-Spectrum β-Lactamase Resistance from Pig Farms to the Surrounding

Environment. *Int J Environ Res Public Health*. 2015;12(4):4203-4213. doi:10.3390/ijerph120404203

373. Ho PL, Liu MCJ, Lo WU, et al. Prevalence and characterization of hybrid blaCTX-M among *Escherichia coli* isolates from livestock and other animals. *Diagn Microbiol Infect Dis*. 2015;82(2):148-153. doi:10.1016/j.diagmicrobio.2015.02.010

374. Schmithausen RM, Schulze-Geisthoevel SV, Stemmer F, et al. Analysis of transmission of MRSA and ESBL-E among pigs and farm personnel. *Plos One*. 2015;10(9):e0138173. doi:10.1371/journal.pone.0138173

375. Hammerum AM, Larsen J, Andersen VD, et al. Characterization of extended-spectrum beta -lactamase (ESBL)-producing *Escherichia coli* obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third- or fourth-generation cephalosporins. *J Antimicrob Chemother*. 2014;69(10):2650–2657.

376. Mandakini R, Dutta TK, Chingtham S, et al. ESBL-producing Shiga-toxigenic *E. coli* (STEC) associated with piglet diarrhoea in India. *Trop Anim Health Prod*. 2014;47(2):377–381. doi:10.1007/s11250-014-0731-1

377. Cortés P, Blanc V, Mora A, et al. Isolation and Characterization of Potentially Pathogenic Antimicrobial-Resistant *Escherichia coli* Strains from Chicken and Pig Farms in Spain. *Appl Environ Microbiol*. 2010;76(9):2799–2805. doi:10.1128/AEM.02421-09

378. Escudero E, Vinue L, Teshager T, Torres C, Moreno MA. Resistance mechanisms and farm-level distribution of fecal *Escherichia coli* isolates resistant to extended-spectrum cephalosporins in pigs in Spain. *Res Vet Sci*. 2010;88(1):83–87. doi:10.1016/j.rvsc.2009.05.021

379. Tseng SP, Wang SF, Kuo CY, et al. Characterization of fosfomycin resistant extended-spectrum beta-lactamase-producing *Escherichia coli* isolates from human and pig in Taiwan. *PLoS ONE*. 2015;10(8):1–14. doi:10.1371/journal.pone.0135864

380. Dang STT, Bortolaia V, Tran NT, Le HQ, Dalsgaard A. Cephalosporin-resistant *Escherichia coli* isolated from farm workers and pigs in northern Vietnam. *Trop Med Int Health*. 2018;23(4):415–424. doi:10.1111/tmi.13054

381. Gousia P, Economou V, Sakkas H, Leveidiotou S, Papadopoulou C. Antimicrobial resistance of major foodborne pathogens from major meat products. *Foodborne Pathog Dis*. 2011;8(1):27–38. doi:10.1089/fpd.2010.0577

382. Pruthvishree B, Vinodh Kumar O, Sinha D, et al. Spatial molecular epidemiology of carbapenem-resistant and New Delhi metallo beta-lactamase (blaNDM) -producing *Escherichia coli* in the piglets of organized farms in India. *J Appl*

Microbiol. 2017;122(6):1537–1546. doi:10.1111/jam.13455

383. Boyen F, Vangroenweghe F, Butaye P, et al. Disk prediffusion is a reliable method for testing colistin susceptibility in porcine *E. coli* strains. *Vet Microbiol*. 2010;144(3-4):359–362. doi:10.1016/j.vetmic.2010.01.010

384. Morales AS, de Araujo JF, Gomes VT de M, et al. Colistin resistance in *Escherichia coli* and *Salmonella enterica* strains isolated from swine in Brazil. *Sci World J*. 2012;2012.

385. Costa MM, Drescher G, Maboni F, et al. Virulence factors, antimicrobial resistance, and plasmid content of *Escherichia coli* isolated in swine commercial farms. *Arq Bras Med Veterinária E Zootec*. 2010;62(1):30–36. doi:10.1590/S0102-09352010000100004

386. da Costa MM, Drescher G, Maboni F, et al. Virulence factors and antimicrobial resistance of *Escherichia* coli isolated from urinary tract of swine in southern of Brazil. *Braz J Microbiol*. 2008;39(4):741–743.

387. Malgarin CM, Takeuti KL. Virulence Factors and Antimicrobial Resistance Profile of *Escherichia coli* Isolated from Nursery Piglets and Drinking Water. *Acta Sci Vet*. 2018;46(1):6. doi:10.22456/1679-9216.81810

388. Lyutskanov M, Urumova V. Etiology, epidemiological monitoring and therapeutic control of bacterial enteritis among suckling pigs in Bulgaria. *Bulg J Vet Med*. 2010;13(4):227–234.

389. Aarestrup FM, Bager F, Jensen NE, Madsen M, Meyling A, Wegener HC. Resistance to antimicrobial agents used for animal therapy in pathogenic-, zoonotic- and indicator bacteria isolated from different food animals in Denmark: A baseline study for the Danish Integrated Antimicrobial Resistance Monitoring Programme (DANMAP). *APMIS Acta Pathol Microbiol Immunol Scand*. 1998;106:745–770. doi:10.1111/j.1699-0463.1998.tb00222.x

390. Belloc C, Nam Lam D, Laval A. Low occurrence of colistin-resistant *Escherichia coli* in faecal content of pigs in French commercial herds. *Rev Médecine Vét*. 2008;159(12):634–637.

391. Kanai H, Hashimoto H, Mitsuhashi S. Drug resistance and conjugative R plasmids in fecal *Escherichia coli* strains isolated from healthy younger animals (chickens, piglets, calves) and children. *Microbiol Immunol*. 1983;27(12):1031–1041. doi:10.7589/0090-3558-31.3.352

392. Asai T, Kojima A, Harada K, Ishihara K, Takahashi T, Tamura Y. Correlation between the usage volume of veterinary therapeutic antimicrobials and resistance in *Escherichia coli* isolated from the feces of food-producing animals in Japan. *Jpn J Infect Dis*. 2005;58(6):369–372.

393. Harada K, Asai T, Kojima A, Oda C, Ishihara K, Takahashi T. Antimicrobial susceptibility of pathogenic *Escherichia coli* isolated from sick cattle and pigs in Japan. *J Vet Med Sci*. 2005;67(10):999–1003. doi:10.1292/jvms.67.999

394. Ahn YT, Park KY, Rha SS. Frequencies of urinary tract infection in sows in Korea. In: *The 6th Asian Pig Veterinary Society Congress Ho Chi Minh City, Vietnam*. ; 2013:123.

395. Olaitan AO, Thongmalayvong B, Akkhavong K, et al. Clonal transmission of a colistin-resistant *Escherichia coli* from a domesticated pig to a human in Laos. *J Antimicrob Chemother*. 2015;70(12):3402–3404. doi:10.1093/jac/dkv252

396. Quesada A, Concepción Porrero M, Téllez S, Palomo G, García M, Domínguez L. Polymorphism of genes encoding PmrAB in colistin-resistant strains of *Escherichia coli* and *Salmonella enterica* isolated from poultry and swine. *J Antimicrob Chemother*. 2015;70(1):71–74. doi:10.1093/jac/dku320

397. Moredo FA, Piñeyro PE, Márquez GC, et al. Enterotoxigenic *Escherichia coli* Subclinical Infection in Pigs: Bacteriological and Genotypic Characterization and Antimicrobial Resistance Profiles. *Foodborne Pathog Dis*. 2015;12(8):704-711. doi:10.1089/fpd.2015.1959

398. Pantozzi FL, Ibar MP, Nievas VF, Vigo GB, Moredo FA, Giacoboni GI. Wild-type minimal inhibitory concentration distributions in bacteria of animal origin in Argentina. *Rev Argent Microbiol*. 2014;46(1):34-40. doi:10.1016/S0325-7541(14)70045-8

399. Bettelheim KA. Antibiotic resistance among verocytotoxigenic *Escherichia coli* (VTEC) and non-VTEC isolated from domestic animals and humans. *J Med Microbiol*. 2003;52(2):155-162. doi:10.1099/jmm.0.04903-0

400. Borges CA, Beraldo LG, Maluta RP, et al. Shiga Toxigenic and Atypical Enteropathogenic *Escherichia coli* in the Feces and Carcasses of Slaughtered Pigs. *Foodborne Pathog Dis*. 2012;9(12):1119-1125. doi:10.1089/fpd.2012.1206

401. Hariharan H, Heaney S, Bryenton J, Daley J. Observations on production of hemolysin, heat-labile enterotoxin and antimicrobial drug resistance among enterotoxigenic *Escherichia coli* from pigs. *Comp Immunol Microbiol Infect Dis*. 1992;15(4):229-234. doi:10.1016/0147-9571(92)90001-8

402. Ludwig A, Berthiaume P, Boerlin P, Gow S, Léger D, Lewis FI. Identifying associations in *Escherichia coli* antimicrobial resistance patterns using additive Bayesian networks. *Prev Vet Med*. 2013;110(1):64-75. doi:10.1016/j.prevetmed.2013.02.005

403. Lapierre L, Cornejo J, Borie C, Toro C, San Martín B. Genetic Characterization of Antibiotic Resistance Genes Linked to Class 1 and Class 2 Integrons in

Commensal Strains of *Escherichia coli* Isolated from Poultry and Swine. *Microb Drug Resist*. 2008;14(4):265-272. doi:10.1089/mdr.2008.0810

404. Lei T, Tian W, He L, et al. Antimicrobial resistance in *Escherichia coli* isolates from food animals, animal food products and companion animals in China. *Vet Microbiol*. 2010;146(1-2):85-89. doi:10.1016/j.vetmic.2010.04.025

405. Yassin AK, JianSen G, Kelly P, et al. Antimicrobial resistance in clinical *Escherichia coli* isolates from poultry and livestock, China. *Plos One*. 2017;12(9):e0185326. doi:10.1371/journal.pone.0185326

406. Asrestrup FM, Jensen NE, Jorsal SE, Neilsen TK. Emergence of resistance to fluoroquinolones among bacteria causing infections in food animals in Denmark. *Vet Rec*. 2000;146(3):76-78. doi:10.1136/vr.146.3.76

407. FINRES. *Bacterial Resistance to Antimicrobial Agents in Finland (1999)*.; 1999. https://www.evira.fi/elaimet/elainten-terveys-ja-elaintaudit/laakitseminen/antibioottiresistenssin-seuranta/finres-vet-raporit/.

408. Shome BR, Rahman M, Murugkar HV, et al. S. Plasmid diversity in *Escherichia coli* strains isolated from piglet diarrhoea. *Indian J Ani Sci.* 2005; 75(2):3.

409. Roychoudhury P, Saikia GK, Rahman H. Serotyping antibiogram of *Escherichia coli* isolated from diarrhoeic piglets. *Indian J Ani Sci.* 2004; 74(1):2.

410. Rajkhowa S, Sarma DK. Prevalence and antimicrobial resistance of porcine O157 and non-O157 Shiga toxin-producing *Escherichia coli* from India. *Trop Anim Health Prod*. 2014;46(6):931-937. doi:10.1007/s11250-014-0587-4

411. Wang L, Nakamura H, Kage-Nakadai E, Hara-Kudo Y, Nishikawa Y. Comparison by multilocus variable-number tandem repeat analysis and antimicrobial resistance among atypical enteropathogenic *Escherichia coli* strains isolated from food samples and human and animal faecal specimens. *J Appl Microbiol*. 2017;122(1):268-278. doi:10.1111/jam.13322

412. Ružauskas M, Šiugždinien R, Daugelavi R. The situation of antimicrobial resistance of enteric bacteria isolated from animal origin to quinolones and fluoroquinolones. *Vet IR Zootech*. 2010:8.

413. Šeputien V, Povilonis J, Ružauskas M, Virgailis M, Žlabys P, Sužied E. Quinolone resistance among *Salmonella enterica* and *Escherichia coli* in Lithuania. *Biologija*. 2006, 3:74-78

414. Nijsten R, London N, van den Bogaard A, Stobberingh E. Antibiotic resistance among *Escherichia coli* isolated from faecal samples of pig farmers and pigs. *J Antimicrob Chemother*. 1996;37(6):1131-1140. doi:10.1093/jac/37.6.1131

415. van den Bogaard AEJM. Antimicrobial resistance in pig faecal samples from The Netherlands (five abattoirs) and Sweden. *J Antimicrob Chemother*. 2000;45(5):663-671. doi:10.1093/jac/45.5.663

416. Nulsen M, Mor M, Lawton D. Antibiotic resistance among indicator bacteria isolated from healthy pigs in New Zealand. *N Z Vet J*. 2008;56(1):29-35. doi:10.1080/00480169.2008.36801

417. Gavrovic M, Asanin R, Misic D, Jezdimirovic M, Zutic M. Investigation of the sensitivity of *E. coli* strains isolated from domestic animals to antibiotics and hemiotherapeutics in vitro. *Acta Vet (Beogr)*. 2011;61(1):21-31. doi:10.2298/AVB1101021G

418. Garau J, Xercavins M, Rodríguez-Carballeira M, et al. Emergence and Dissemination of Quinolone-Resistant *Escherichia coli* in the Community. *Antimicrob Agents Chemother*. 1999;43(11):2736-2741. doi:10.1128/AAC.43.11.2736

419. Miranda JM, Vázquez BI, Fente CA, Barros-Velázquez J, Cepeda A, Franco Abuín CM. Antimicrobial resistance in *Escherichia coli* strains isolated from organic and conventional pork meat: A comparative survey. *Eur Food Res Technol*. 2008;226(3):371-375. doi:10.1007/s00217-006-0547-y

420. Stephan R, Schumacher S. Resistance patterns of non-O157 Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from animals, food and asymptomatic human carriers in Switzerland. *Lett Appl Microbiol*. 2001;32(2):114–117. doi:10.1046/j.1472-765x.2001.00867.x

421. Lanz R, Kuhnert P, Boerlin P. Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. *Vet Microbiol*. 2003;91(1):73-84. doi:10.1016/S0378-1135(02)00263-8

422. Prapasarakul N, Tummaruk P, Niyomtum W, Tripipat T, Serichantalergs O. Virulence genes and antimicrobial susceptibilities of hemolytic and nonhemolytic *Escherichia coli* isolated from post-weaning piglets in central Thailand. *J Vet Med Sci*. 2010;72(12):1602–1608. doi:10.1292/jvms.10-0124

423. Laohasinnarong D, Thanachotsirivibul W, Limrungsukho W, Gronsang D. Antimicrobial susceptibility patterns of *Escherichia coli* in diarrheal piglet fecal samples: Using continuous medicated feed and geographical variation. *J Appl Anim Sci*. 2012;5(1):18–24.

424. Okello E, Moonens K, Erume J, De Greve H. Enterotoxigenic *Escherichia coli* strains are highly prevalent in Ugandan piggeries but disease outbreaks are masked by antibiotic prophylaxis. *Trop Anim Health Prod*. 2015;47(1):117-122. doi:10.1007/s11250-014-0694-2

425. Van TTH, Chin J, Chapman T, Tran LT, Coloe PJ. Safety of raw meat and shellfish in Vietnam: An analysis of *Escherichia coli* isolations for antibiotic resistance and virulence genes. *Int J Food Microbiol*. 2008;124(3):217-223. doi:10.1016/j.ijfoodmicro.2008.03.029

426. Hart WS, Heuzenroeder MW, Barton MD. Antimicrobial Resistance in *Campylobacter* spp., *Escherichia coli* and *Enterococci* Associated with Pigs in Australia. *J Vet Med Ser B*. 2004;51(5):216-221. doi:10.1111/j.1439-0450.2004.00760.x

427. Mayrhofer S, Paulsen P, Smulders FJM, Hilbert F. Antimicrobial Resistance in Commensal *Escherichia coli* Isolated from Muscle Foods as Related to the Veterinary Use of Antimicrobial Agents in Food-Producing Animals in Austria. *Microb Drug Resist*. 2006;12(4):278-283. doi:10.1089/mdr.2006.12.278

428. Read SC, Gyles CL, Clarke RC, Lior H, McEwen S. Prevalence of verocytotoxigenic *Escherichia coli* in ground beef, pork, and chicken in southwestern Ontario. *Epidemiol Infect*. 1990;105(1):11-20. doi:10.1017/S0950268800047592

429. AalbaeK B, Rasmussen J, Nielsen B, Olsen JE. Prevalence of antibiotic-resistant *Escherichia coli* in Danish pigs and cattle. *APMIS*. 1991;99(7-12):1103-1110. doi:10.1111/j.1699-0463.1991.tb01307.x

430. Jorgensen ST, Poulsen A-L. Antibiotic Resistance and Hly Plasmids in Serotypes of *Escherichia coli* Associated with Porcine Enteric Disease. *Antimicrob Agents Chemother*. 1976;9(1):6-10. doi:10.1128/AAC.9.1.6

431. Adetosoye AI. Infective drug resistance among *Escherichia coli* isolated from clinically healthy domestic livestock. *Vet Microbiol*. 1980;5(4):333-342. doi:10.1016/0378-1135(80)90032-2

432. Smith HW. The incidence of infective drug resistance in strains of Escherichia coli isolated from diseased human beings and domestic animals. *Epidemiology & Infection*. 1966; 64(4):465-474.

433. Smith HW. Antibiotic-Resistant *Escherichia coli* in Market Pigs in 1956-1979: The Emergence of Organisms with Plasmid-Borne Trimethoprim Resistance. *J Hyg*. 1980;84(3):467-477.

434. Smith HW, Lovell MA. *Escherichia coli* resistant to tetracyclines and to other antibiotics in the faeces of U.K. chickens and pigs in 1980. *J Hyg*. 1981;87(3):477-483. doi:10.1017/S0022172400069722

435. Cooke EM, Breaden AL, Shooter RA, O'Farrell SM. Antibiotic sensitivity of *Escherichia coli* isolated from animals, food, hospital patients and normal people. *The Lancet*. 1971;298(7714):8-10. doi:10.1016/S0140-6736(71)90004-3

436. Ngoma M, Suzuki A, Takashima I, Sato G. Antibiotic resistance of *Escherichia coli* and Salmonella from apparently healthy slaughtered cattle and pigs, and diseased animals in Zambia. *Jpn J Vet Res*. 1993;41(1):1–10.

437. Blanc V, Mesa R, Saco M, et al. ESBL- and plasmidic class C β-lactamase-producing *E. coli* strains isolated from poultry, pig and rabbit farms. *Vet Microbiol*. 2006;118(3-4):299-304. doi:10.1016/j.vetmic.2006.08.002

438. Wegener HC. Antibiotics in animal feed and their role in resistance development. *Curr Opin Microbiol*. 2003;6(5):439-445. doi:10.1016/j.mib.2003.09.009

439. FDA. 2017 Summary Report On Antimicrobials Sold or Distributed for Use in Food-Producing Animals. https://www.fda.gov/animal-veterinary/cvm-updates/fda-releases-annual-summary-report-antimicrobials-sold-or-distributed-2017-use-food-producing.

440. Leimbach A, Hacker J, Dobrindt U. E. coli as an All-Rounder: The Thin Line Between Commensalism and Pathogenicity. In: Dobrindt U, Hacker JH, Svanborg C, eds. *Between Pathogenicity and Commensalism*. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013:3-32. doi:10.1007/82_2012_303

441. Glass-Kaastra SK, Pearl DL, Reid-Smith RJ, et al. Surveillance of antimicrobial resistance in clinical isolates of *Pasteurella multocida* and *Streptococcus suis* from Ontario swine. *Can J Vet Res*. 2014;78(4):241-249.

442. Ludecke D. Sjstats: Collection of convenient functions for common statistical computations. https://strengejacke.github.io/sjstats/. Accessed 20 August, 2019.

443. Kadykalo SV, Anderson MEC, Alsop JE. Passive surveillance of antimicrobial resistance in *Salmonella* and *Escherichia coli* isolates from Ontario livestock, 2007-2015. *Can Vet J Rev Veterinaire Can*. 2018;59(6):617-622.

444. Luppi A, Bonilauri P, Dottori M, et al. Antimicrobial Resistance of F4+ *Escherichia Coli* Isolated from Swine in Italy. *Transbound Emerg Dis*. 2015;62(1):67-71. doi:10.1111/tbed.12081

445. Hanon JB, Jaspers S, Butaye P, et al. A trend analysis of antimicrobial resistance in commensal *Escherichia coli* from several livestock species in Belgium (2011–2014). *Prev Vet Med*. 2015;122(4):443-452. doi:10.1016/j.prevetmed.2015.09.001

446. Trouchon T, Lefebvre S. A Review of Enrofloxacin for Veterinary Use. *Open J Vet Med*. 2016;6(2):40-58. doi:10.4236/ojvm.2016.62006

447. Hong S, Rovira A, Davies P, et al. Serotypes and Antimicrobial Resistance in *Salmonella enterica* Recovered from Clinical Samples from Cattle and Swine in Minnesota, 2006 to 2015. *PLOS ONE*. 2016;11(12):e0168016. doi:10.1371/journal.pone.0168016

448. Cantón R, Ruiz-Garbajosa P. Co-resistance: An opportunity for the bacteria and resistance genes. *Curr Opin Pharmacol*. 2011;11(5):477-485. doi:10.1016/j.coph.2011.07.007

449. Sundqvist M, Geli P, Andersson DI, et al. Little evidence for reversibility of trimethoprim resistance after a drastic reduction in trimethoprim use. *J Antimicrob Chemother*. 2010;65(2):350-360. doi:10.1093/jac/dkp387

450. Awosile B, McClure J, Sanchez J, Rodriguez-Lecompte JC, Keefe G, Heider LC. *Salmonella enterica* and extended-spectrum cephalosporin-resistant *Escherichia coli* recovered from Holstein dairy calves from 8 farms in New Brunswick, Canada. *J Dairy Sci*. 2018;101(4):3271-3284. doi:10.3168/jds.2017-13277

451. Mazloom R, Jaberi-Douraki M, Comer JR, Volkova V. Potential Information Loss Due to Categorization of Minimum Inhibitory Concentration Frequency Distributions. *Foodborne Pathog Dis*. 2017;15(1):44-54. doi:10.1089/fpd.2017.2301

452. Sweeney MT, Lubbers BV, Schwarz S, Watts JL. Applying definitions for multidrug resistance, extensive drug resistance and pandrug resistance to clinically significant livestock and companion animal bacterial pathogens. *J Antimicrob Chemother*. 2018;73(6):1460-1463. doi:10.1093/jac/dky043

453. Food and Drug Administration. NARMS now. https://www.fda.gov/animal-veterinary/national-antimicrobial-resistance-monitoring-system/narms-now-integrated-data. Accessed July 28, 2019.

454. Tyson GH, Tate HP, Zhao S, et al. Identification of Plasmid-Mediated Quinolone Resistance in *Salmonella* Isolated from Swine Ceca and Retail Pork Chops in the United States. *Antimicrob Agents Chemother*. 2017;61(10):e01318-17, /aac/61/10/e01318-17.atom. doi:10.1128/AAC.01318-17

455. Fablet C, Marois-Créhan C, Simon G, et al. Infectious agents associated with respiratory diseases in 125 farrow-to-finish pig herds: A cross-sectional study. *Vet Microbiol*. 2012;157(1):152-163. doi:10.1016/j.vetmic.2011.12.015

456. Brockmeier SL, Halbur PG, Thacker EL. *Porcine Respiratory Disease Complex*. ASM Press; 2002. https://www.ncbi.nlm.nih.gov/books/NBK2481/. Accessed July 27, 2019.

457. MacInnes JI, Desrosiers R. Agents of the "suis-ide diseases" of swine: *Actinobacillus suis*, *Haemophilus parasuis*, and *Streptococcus suis*. *Can J Vet Res*. 1999;63(2):83-89.

458. Oliveira Filho JX de, Morés MAZ, Rebellato R, et al. Pathogenic variability among *Pasteurella multocida* type A isolates from Brazilian pig farms. *BMC Vet Res*. 2018;14(1):244. doi:10.1186/s12917-018-1565-2

459. Opriessnig T, Giménez-Lirola LG, Halbur PG. Polymicrobial respiratory disease in pigs. *Anim Health Res Rev*. 2011;12(2):133-148. doi:10.1017/S1466252311000120

460. Varela NP, Gadbois P, Thibault C, Gottschalk M, Dick P, Wilson J. Antimicrobial resistance and prudent drug use for *Streptococcus suis*. *Anim Health Res Rev*. 2013;14(1):68-77. doi:10.1017/S1466252313000029

461. Brean M, Abraham S, Hebart M, Kirkwood RN. Influence of parity of birth and suckled sows on piglet nasal mucosal colonization with *Haemophilus parasuis*. *Can Vet J*. 2016;57(12):1281-1283.

462. MacInnes JI, Gottschalk M, Lone AG, et al. Prevalence of *Actinobacillus pleuropneumoniae, Actinobacillus suis, Haemophilus parasuis, Pasteurella multocida*, and *Streptococcus suis* in representative Ontario swine herds. *Can J Vet Res*. 2008;72(3):242-248.

463. Vötsch D, Willenborg M, Weldearegay YB, Valentin-Weigand P. *Streptococcus suis* – The "Two Faces" of a Pathobiont in the Porcine Respiratory Tract. *Front Microbiol*. 2018;9. doi:10.3389/fmicb.2018.00480

464. Lapointe L, D'Allaire S, Lebrun A, Lacouture S, Gottschalk M. Antibody response to an autogenous vaccine and serologic profile for *Streptococcus suis* capsular type 1/2. *Can J Vet Res*. 2002;66(1):8-14.

465. Macedo N, Rovira A, Torremorell M. *Haemophilus parasuis*: Infection, immunity and enrofloxacin. *Vet Res*. 2015;46(1):128. doi:10.1186/s13567-015-0263-3

466. Lin W-H, Shih H-C, Lin C-F, et al. Molecular serotyping of *Haemophilus parasuis* isolated from diseased pigs and the relationship between serovars and pathological patterns in Taiwan. *PeerJ*. 2018;6:e6017. doi:10.7717/peerj.6017

467. Jiang X, Yang Y, Zhu L, et al. Live *Streptococcus suis* type 5 strain XS045 provides cross-protection against infection by strains of types 2 and 9. *Vaccine*. 2016;34(51):6529-6538. doi:10.1016/j.vaccine.2016.05.003

468. Miani M, Lorenson MS, Guizzo JA, et al. Antimicrobial susceptibility patterns of Brazilian *Haemophilus parasuis* field isolates. *Pesqui Veterinária Bras*. 2017;37(11):1187-1192. doi:10.1590/s0100-736x2017001100001

469. Sweeney MT, Lindeman C, Johansen L, et al. Antimicrobial susceptibility of *Actinobacillus pleuropneumoniae, Pasteurella multocida, Streptococcus suis*, and *Bordetella bronchiseptica* isolated from pigs in the United States and Canada, 2011 to 2015. *J Swine Health Prod*. 2017;25(3):15.

470. Toutain P-L, Bousquet-Mélou A, Damborg P, et al. En Route towards European Clinical Breakpoints for Veterinary Antimicrobial Susceptibility Testing: A Position Paper Explaining the VetCAST Approach. *Front Microbiol*. 2017;8.

doi:10.3389/fmicb.2017.02344

471. Clinical and Laboraory Standards Institute (CLSI). *Performance Standards for Antimcirobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals VET08*. 4th ed. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania-19087, USA; 2018.

472. Couto N, Monchique C, Belas A, Marques C, Gama LT, Pomba C. Trends and molecular mechanisms of antimicrobial resistance in clinical staphylococci isolated from companion animals over a 16 year period. *J Antimicrob Chemother*. 2016;71(6):1479-1487. doi:10.1093/jac/dkw029

473. Fox-Lewis A, Takata J, Miliya T, et al. Antimicrobial Resistance in Invasive Bacterial Infections in Hospitalized Children, Cambodia, 2007–2016. *Emerg Infect Dis*. 2018;24(5):841-851. doi:10.3201/eid2405.171830

474. Brant R. Assessing Proportionality in the Proportional Odds Model for Ordinal Logistic Regression. *Biometrics*. 1990;46(4):1171-1178. doi:10.2307/2532457

475. Byrt T, Bishop J, Carlin JB. Bias, prevalence and kappa. *J Clin Epidemiol*. 1993;46(5):423-429. doi:10.1016/0895-4356(93)90018-V

476. Hernandez-Garcia J, Wang J, Restif O, et al. Patterns of antimicrobial resistance in *Streptococcus suis* isolates from pigs with or without streptococcal disease in England between 2009 and 2014. *Vet Microbiol*. 2017;207:117-124. doi:10.1016/j.vetmic.2017.06.002

477. Callens BF, Haesebrouck F, Maes D, Butaye P, Dewulf J, Boyen F. Clinical Resistance and Decreased Susceptibility in *Streptococcus suis* Isolates from Clinically Healthy Fattening Pigs. *Microb Drug Resist*. 2013;19(2):146-151. doi:10.1089/mdr.2012.0131

478. Wisselink HJ, Veldman KT, Van den Eede C, Salmon SA, Mevius DJ. Quantitative susceptibility of *Streptococcus suis* strains isolated from diseased pigs in seven European countries to antimicrobial agents licenced in veterinary medicine. *Vet Microbiol*. 2006;113(1-2):73-82. doi:10.1016/j.vetmic.2005.10.035

479. Vela AI, Moreno MiguelA, Cebolla JA, et al. Antimicrobial susceptibility of clinical strains of *Streptococcus suis* isolated from pigs in Spain. *Vet Microbiol*. 2005;105(2):143-147. doi:10.1016/j.vetmic.2004.10.009

480. van Hout J, Heuvelink A, Gonggrijp M. Monitoring of antimicrobial susceptibility of *Streptococcus suis* in the Netherlands, 2013–2015. *Vet Microbiol*. 2016;194:5-10. doi:10.1016/j.vetmic.2016.03.014

481. O'Dea MA, Laird T, Abraham R, et al. Examination of Australian *Streptococcus Suis* Isolates From Clinically Affected Pigs in a Global Context and the Genomic

Characterisation of ST1 as a Predictor of Virulence. Microbiology; 2018. doi:10.1101/375543

482. Li L-L, Liao X-P, Sun J, et al. Antimicrobial Resistance, Serotypes, and Virulence Factors of *Streptococcus suis* Isolates from Diseased Pigs. *Foodborne Pathog Dis*. 2012;9(7):583-588. doi:10.1089/fpd.2011.1106

483. Dayao DAE, Gibson JS, Blackall PJ, Turni C. Antimicrobial resistance in bacteria associated with porcine respiratory disease in Australia. *Vet Microbiol*. 2014;171(1-2):232-235. doi:10.1016/j.vetmic.2014.03.014

484. Tang X, Zhao Z, Hu J, et al. Isolation, Antimicrobial Resistance, and Virulence Genes of *Pasteurella multocida* Strains from Swine in China. *J Clin Microbiol*. 2009;47(4):951-958. doi:10.1128/JCM.02029-08

485. Yeh JC, Lo DY, Chang S-K, Chou CC, Kuo HC. Antimicrobial susceptibility, serotypes and genotypes of *Pasteurella multocida* isolates associated with swine pneumonia in Taiwan. *Vet Rec*. 2017;181(12):323-323. doi:10.1136/vr.104023

486. Schwarz S, Silley P, Simjee S, et al. Editorial: Assessing the antimicrobial susceptibility of bacteria obtained from animals. *J Antimicrob Chemother*. 2010;65(4):601-604. doi:10.1093/jac/dkq037

487. Brogden S, Pavlović A, Tegeler R, Kaspar H, De Vaan N, Kehrenberg C. Antimicrobial susceptibility of *Haemophilus parasuis* isolates from Germany by use of a proposed standard method for harmonized testing. *Vet Microbiol*. 2018;217:32-35. doi:10.1016/j.vetmic.2018.02.017

488. Dayao DAE, Kienzle M, Gibson JS, Blackall PJ, Turni C. Use of a proposed antimicrobial susceptibility testing method for *Haemophilus parasuis*. *Vet Microbiol*. 2014;172(3-4):586-589. doi:10.1016/j.vetmic.2014.06.010

489. Zhao Y, Wang Q, Li J, Lin X, Huang X, Fang B. Epidemiology of *Haemophilus parasuis* isolates from pigs in China using serotyping, antimicrobial susceptibility, biofilm formation and ERIC-PCR genotyping. *PeerJ*. 2018;6:e5040. doi:10.7717/peerj.5040

490. Cusack TP, Ashley EA, Ling CL, et al. Impact of CLSI and EUCAST breakpoint discrepancies on reporting of antimicrobial susceptibility and AMR surveillance. *Clin Microbiol Infect*. 2019;25(7):910-911. doi:10.1016/j.cmi.2019.03.007

491. Prüller S, Turni C, Blackall PJ, et al. Towards a Standardized Method for Broth Microdilution Susceptibility Testing of *Haemophilus parasuis*. Richter SS, ed. *J Clin Microbiol*. 2017;55(1):264-273. doi:10.1128/JCM.01403-16

492. Yongkiettrakul S, Maneerat K, Arechanajan B, et al. Antimicrobial susceptibility of *Streptococcus suis* isolated from diseased pigs, asymptomatic pigs, and human

patients in Thailand. *BMC Vet Res*. 2019;15(1):5. doi:10.1186/s12917-018-1732-5

493. Zhou X, Xu X, Zhao Y, et al. Distribution of antimicrobial resistance among different serovars of *Haemophilus parasuis* isolates. *Vet Microbiol*. 2010;141(1-2):168-173. doi:10.1016/j.vetmic.2009.05.012

494. de la Fuente AJM, Tucker AW, Navas J, Blanco M, Morris SJ, Gutiérrez-Martín CB. Antimicrobial susceptibility patterns of *Haemophilus parasuis* from pigs in the United Kingdom and Spain. *Vet Microbiol*. 2007;120(1-2):184-191. doi:10.1016/j.vetmic.2006.10.014

495. Kucerova Z, Hradecka H, Nechvatalova K, Nedbalcova K. Antimicrobial susceptibility of *Actinobacillus pleuropneumoniae* isolates from clinical outbreaks of porcine respiratory diseases. *Vet Microbiol*. 2011;150(1-2):203-206. doi:10.1016/j.vetmic.2011.01.016

496. Carmo LP, Nielsen LR, Alban L, Müntener CR, Schüpbach-Regula G, Magouras I. Comparison of Antimicrobial Consumption Patterns in the Swiss and Danish Cattle and Swine Production (2007–2013). *Front Vet Sci*. 2017;4. doi:10.3389/fvets.2017.00026

497. Neuert S, Nair S, Day MR, et al. Prediction of Phenotypic Antimicrobial Resistance Profiles From Whole Genome Sequences of Non-typhoidal *Salmonella enterica*. *Front Microbiol*. 2018;9. doi:10.3389/fmicb.2018.00592

498. Edirmanasinghe R, Finley R, Parmley EJ, et al. A Whole-Genome Sequencing Approach To Study Cefoxitin-Resistant *Salmonella enterica* Serovar Heidelberg Isolates from Various Sources. *Antimicrob Agents Chemother*. 2017;61(4):e01919-16, /aac/61/4/e01919-16.atom. doi:10.1128/AAC.01919-16

499. Magouras I, Carmo LP, Stärk KDC, Schüpbach-Regula G. Antimicrobial Usage and -Resistance in Livestock: Where Should We Focus? *Front Vet Sci*. 2017;4. doi:10.3389/fvets.2017.00148

500. Frye JG, Jackson CR. Genetic mechanisms of antimicrobial resistance identified in *Salmonella enterica, Escherichia coli,* and *Enteroccocus* spp. Isolated from U.S. food animals. *Front Microbiol*. 2013;4. doi:10.3389/fmicb.2013.00135

501. Wittum TE, Mollenkopf DF, Erdman MM. Detection of *Salmonella enterica* Isolates Producing CTX-M Cephalosporinase in U.S. Livestock Populations. *Appl Environ Microbiol*. 2012;78(20):7487-7491. doi:10.1128/AEM.01682-12

502. Elnekave E, Hong SL, Lim S, et al. Circulation of Plasmids Harboring Resistance Genes to Quinolones and/or Extended-Spectrum Cephalosporins in Multiple *Salmonella enterica* Serotypes from Swine in the United States. *Antimicrob Agents Chemother*. 2019;63(4):e02602-18. doi:10.1128/AAC.02602-18

503. Elnekave E, Hong S, Mather AE, et al. *Salmonella enterica* Serotype 4,[5],12:i:- in Swine in the United States Midwest: An Emerging Multidrug-Resistant Clade. *Clin Infect Dis*. 2018;66(6):877-885. doi:10.1093/cid/cix909

504. Codjoe F, Donkor E. Carbapenem Resistance: A Review. *Med Sci*. 2017;6(1):1. doi:10.3390/medsci6010001

505. Feng S, Shen C, Chen H, et al. Co-production of *mcr-1* and NDM-5 in Escherichia coli isolated from a colonization case of inpatient. *Infect Drug Resist*. 2018;11:1157-1161. doi:10.2147/IDR.S171164

506. Birgy A, Madhi F, Hogan J, et al. CTX-M-55-, *mcr-1*-, and *fosA*-Producing Multidrug-Resistant *Escherichia coli* Infection in a Child in France. *Antimicrob Agents Chemother*. 2018;62(4):e00127-18, /aac/62/4/e00127-18.atom. doi:10.1128/AAC.00127-18

507. Silver LL. Fosfomycin: Mechanism and Resistance. *Cold Spring Harb Perspect Med*. 2017;7(2):a025262. doi:10.1101/cshperspect.a025262

508. Bankevich A, Nurk S, Antipov D, et al. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *J Comput Biol*. 2012;19(5):455-477. doi:10.1089/cmb.2012.0021

509. Larsen MV, Cosentino S, Rasmussen S, et al. Multilocus Sequence Typing of Total-Genome-Sequenced Bacteria. *J Clin Microbiol*. 2012;50(4):1355-1361. doi:10.1128/JCM.06094-11

510. Zankari E, Hasman H, Cosentino S, et al. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother*. 2012;67(11):2640-2644. doi:10.1093/jac/dks261

511. Carattoli A, Zankari E, García-Fernández A, et al. *In Silico* Detection and Typing of Plasmids using PlasmidFinder and Plasmid Multilocus Sequence Typing. *Antimicrob Agents Chemother*. 2014;58(7):3895-3903. doi:10.1128/AAC.02412-14

512. Seemann T. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics*. 2014;30(14):2068-2069. doi:10.1093/bioinformatics/btu153

513. Page AJ, Cummins CA, Hunt M, et al. Roary: Rapid large-scale prokaryote pan genome analysis. *Bioinformatics*. 2015;31(22):3691-3693. doi:10.1093/bioinformatics/btv421

514. Treangen TJ, Ondov BD, Koren S, Phillippy AM. Rapid Core-Genome Alignment and Visualization for Thousands of Intraspecific Microbial Genomes. Bioinformatics; 2014. doi:10.1101/007351

515. Stamatakis A. RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 2014;30(9):1312-1313. doi:10.1093/bioinformatics/btu033

516. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: Recent updates and new developments. *Nucleic Acids Res*. 2019;47(W1):W256-W259. doi:10.1093/nar/gkz239

517. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. *PLOS Comput Biol*. 2017;13(6):e1005595. doi:10.1371/journal.pcbi.1005595

518. Varani AM, Siguier P, Gourbeyre E, Charneau V, Chandler M. ISsaga is an ensemble of web-based methods for high throughput identification and semi-automatic annotation of insertion sequences in prokaryotic genomes. *Genome Biol*. 2011;12(3):R30. doi:10.1186/gb-2011-12-3-r30

519. Galata V, Fehlmann T, Backes C, Keller A. PLSDB: A resource of complete bacterial plasmids. *Nucleic Acids Res*. 2019;47(D1):D195-D202. doi:10.1093/nar/gky1050

520. Alikhan N-F, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): Simple prokaryote genome comparisons. *BMC Genomics*. 2011;12(1):402. doi:10.1186/1471-2164-12-402

521. Langmead B, Salzberg SL. Fast gapped-read alignment with Bowtie 2. *Nat Methods*. 2012;9(4):357-359. doi:10.1038/nmeth.1923

522. Roach S, Wallinga D. Commentary on genetic mechanisms of antimicrobial resistance in bacteria from U.S. food animals: ESBLs are here. *Front Microbiol*. 2013;4. doi:10.3389/fmicb.2013.00214

523. Tadesse DA, Li C, Mukherjee S, et al. Whole-Genome Sequence Analysis of CTX-M Containing *Escherichia coli* Isolates from Retail Meats and Cattle in the United States. *Microb Drug Resist*. 2018;24(7):939-948. doi:10.1089/mdr.2018.0206

524. Jiang F, Wu Z, Zheng Y, et al. Genotypes and Antimicrobial Susceptibility Profiles of Hemolytic *Escherichia coli* from Diarrheic Piglets. *Foodborne Pathog Dis*. 2019;16(2):94-103. doi:10.1089/fpd.2018.2480

525. Cummings KJ, Rodriguez-Rivera LD, Norman KN, Ohta N, Scott HM. Identification of a Plasmid-Mediated Quinolone Resistance Gene in *Salmonella* Isolates from Texas Dairy Farm Environmental Samples. *Zoonoses Public Health*. 2017;64(4):305-307. doi:10.1111/zph.12318

526. Tran JH, Jacoby GA, Hooper DC. Interaction of the Plasmid-Encoded Quinolone Resistance Protein *Qnr* with *Escherichia coli* DNA Gyrase. *Antimicrob Agents*

Chemother. 2005;49(1):118-125. doi:10.1128/AAC.49.1.118-125.2005

527. Martínez-Martínez L, Pascual A, García I, Tran J, Jacoby GA. Interaction of plasmid and host quinolone resistance. *J Antimicrob Chemother*. 2003;51(4):1037-1039. doi:10.1093/jac/dkg157

528. Irrgang A, Falgenhauer L, Fischer J, et al. CTX-M-15-Producing *E. coli* Isolates from Food Products in Germany Are Mainly Associated with an IncF-Type Plasmid and Belong to Two Predominant Clonal *E. coli* Lineages. *Front Microbiol*. 2017;8. doi:10.3389/fmicb.2017.02318

529. Falgenhauer L, Imirzalioglu C, Ghosh H, et al. Circulation of clonal populations of fluoroquinolone-resistant CTX-M-15-producing *Escherichia coli* ST410 in humans and animals in Germany. *Int J Antimicrob Agents*. 2016;47(6):457-465. doi:10.1016/j.ijantimicag.2016.03.019

530. Pitout JDD, DeVinney R. Escherichia coli ST131: A multidrug-resistant clone primed for global domination. *F1000Research*. 2017;6. doi:10.12688/f1000research.10609.1

531. Maluta RP, Logue CM, Casas MRT, et al. Overlapped Sequence Types (STs) and Serogroups of Avian Pathogenic (APEC) and Human Extra-Intestinal Pathogenic (ExPEC) *Escherichia coli* Isolated in Brazil. *PLOS ONE*. 2014;9(8):e105016. doi:10.1371/journal.pone.0105016

532. Day MJ, Rodríguez I, van Essen-Zandbergen A, et al. Diversity of STs, plasmids and ESBL genes among *Escherichia coli* from humans, animals and food in Germany, the Netherlands and the UK. *J Antimicrob Chemother*. 2016;71(5):1178-1182. doi:10.1093/jac/dkv485

533. Hasman H, Hammerum AM, Hansen F, et al. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Eurosurveillance*. 2015;20(49):1–5. doi:10.2807/1560-7917.ES.2015.20.49.30085

534. Mellmann A, Bielaszewska M, Köck R, et al. Analysis of Collection of Hemolytic Uremic Syndrome–associated Enterohemorrhagic *Escherichia coli*. *Emerg Infect Dis*. 2008;14(8):1287-1290. doi:10.3201/eid1408.071082

535. Schaufler K, Semmler T, Wieler LH, et al. Clonal spread and interspecies transmission of clinically relevant ESBL-producing *Escherichia coli* of ST410—another successful pandemic clone? *FEMS Microbiol Ecol*. 2016;92(1). doi:10.1093/femsec/fiv155

536. Li J-J, Spychala CN, Hu F, Sheng J-F, Doi Y. Complete Nucleotide Sequences of blaCTX-M-Harboring IncF Plasmids from Community-Associated *Escherichia coli* Strains in the United States. *Antimicrob Agents Chemother*. 2015;59(6):3002-

3007. doi:10.1128/AAC.04772-14

537. McCollister B, Kotter CV, Frank DN, Washburn T, Jobling MG. Whole-Genome Sequencing Identifies In Vivo Acquisition of a blaCTX-M-27-Carrying IncFII Transmissible Plasmid as the Cause of Ceftriaxone Treatment Failure for an Invasive *Salmonella enterica* Serovar Typhimurium Infection. *Antimicrob Agents Chemother*. 2016;60(12):13.

538. Mook P, McCormick J, Bains M, et al. ESBL-Producing and Macrolide-Resistant *Shigella sonnei* Infections among Men Who Have Sex with Men, England, 2015. *Emerg Infect Dis*. 2016;22(11):1948-1952. doi:10.3201/eid2211.160653

539. Sun H, Li S, Xie Z, et al. A novel multidrug resistance plasmid isolated from an *Escherichia coli* strain resistant to aminoglycosides. *J Antimicrob Chemother*. 2012;67(7):1635-1638. doi:10.1093/jac/dks107

540. Ho PL, Lo WU, Wong RCW, et al. Complete sequencing of the FII plasmid pHK01, encoding CTX-M-14, and molecular analysis of its variants among *Escherichia coli* from Hong Kong. *J Antimicrob Chemother*. 2011;66(4):752-756. doi:10.1093/jac/dkr010

541. Chavda KD, Chen L, Fouts DE, et al. Comprehensive Genome Analysis of Carbapenemase-Producing *Enterobacter* spp.: New Insights into Phylogeny, Population Structure, and Resistance Mechanisms. *MBio*. 2016;7(6):e02093-16. doi:10.1128/mBio.02093-16

542. Shi L, Liang Q, Zhan Z, et al. Co-occurrence of 3 different resistance plasmids in a multi-drug resistant *Cronobacter sakazakii* isolate causing neonatal infections. *Virulence*. 2017;9(1):110-120. doi:10.1080/21505594.2017.1356537

543. Chen YT, Liao TL, Liu YM, Lauderdale TL, Yan JJ, Tsai SF. Mobilization of *qnrB2* and *ISCR1* in Plasmids. *Antimicrob Agents Chemother*. 2009;53(3):1235-1237. doi:10.1128/AAC.00970-08

544. Samuelsen Ø, Hansen F, Aasnæs B, et al. Dissemination and Characteristics of a Novel Plasmid-Encoded Carbapenem-Hydrolyzing Class D β-Lactamase, OXA-436, Found in Isolates from Four Patients at Six Different Hospitals in Denmark. *Antimicrob Agents Chemother*. 2017;62(1):e01260-17. doi:10.1128/AAC.01260-17

545. Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile Genetic Elements Associated with Antimicrobial Resistance. *Clin Microbiol Rev*. 2018;31(4):e00088-17. doi:10.1128/CMR.00088-17

546. San Millan A, MacLean RC. Fitness Costs of Plasmids: A Limit to Plasmid Transmission. *Microbiol Spectr*. 2017;5(5). doi:10.1128/microbiolspec.MTBP-0016-2017

547. Johnson TJ, Singer RS, Isaacson RE, et al. In Vivo Transmission of an IncA/C Plasmid in *Escherichia coli* Depends on Tetracycline Concentration, and Acquisition of the Plasmid Results in a Variable Cost of Fitness. *Appl Environ Microbiol*. 2015;81(10):3561-3570. doi:10.1128/AEM.04193-14

548. Buckner MMC, Saw HTH, Osagie RN, et al. Clinically Relevant Plasmid-Host Interactions Indicate that Transcriptional and Not Genomic Modifications Ameliorate Fitness Costs of *Klebsiella pneumoniae* Carbapenemase-Carrying Plasmids. Bonomo RA, ed. *MBio*. 2018;9(2):e02303-17, /mbio/9/2/mBio.02303-17.atom. doi:10.1128/mBio.02303-17

549. Schaufler K, Semmler T, Pickard DJ, et al. Carriage of Extended-Spectrum Beta-Lactamase-Plasmids Does Not Reduce Fitness but Enhances Virulence in Some Strains of Pandemic *E. coli* Lineages. *Front Microbiol*. 2016;7. doi:10.3389/fmicb.2016.00336

550. Carattoli A. Plasmids and the spread of resistance. *Int J Med Microbiol*. 2013;303(6):298-304. doi:10.1016/j.ijmm.2013.02.001

551. García-Fernández A, Carattoli A. Plasmid double locus sequence typing for IncHI2 plasmids, a subtyping scheme for the characterization of IncHI2 plasmids carrying extended-spectrum β-lactamase and quinolone resistance genes. *J Antimicrob Chemother*. 2010;65(6):1155-1161. doi:10.1093/jac/dkq101

552. Ho PL, Lo WU, Yeung MK, et al. Dissemination of pHK01-like incompatibility group IncFII plasmids encoding CTX-M-14 in *Escherichia coli* from human and animal sources. *Vet Microbiol*. 2012;158(1-2):172-179. doi:10.1016/j.vetmic.2012.02.004

553. Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO, Wiedmann M. Identification of Novel Mobilized Colistin Resistance Gene *mcr-9* in a Multidrug-Resistant, Colistin-Susceptible *Salmonella enterica* Serotype Typhimurium Isolate. *MBio*. 2019;10(3). doi:10.1128/mBio.00853-19

554. Rehman MA, Yin X, Persaud-Lachhman MG, Diarra MS. First Detection of a Fosfomycin Resistance Gene, *fosA7*, in Salmonella enterica Serovar Heidelberg Isolated from Broiler Chickens. *Antimicrob Agents Chemother*. 2017;61(8). doi:10.1128/AAC.00410-17

555. Ito R, Mustapha MM, Tomich AD, et al. Widespread Fosfomycin Resistance in Gram-Negative Bacteria Attributable to the Chromosomal *fosA* Gene. Bush K, ed. *MBio*. 2017;8(4):e00749-17, /mbio/8/4/e00749-17.atom. doi:10.1128/mBio.00749-17